THE WASSERMANN TEST

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BY

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PREFACE

The following work upon the Wassermann test has been written at the solicitation of friends who have urged me to place upon record the experience gained from nearly ten years of personal use of this test in the diagnosis and as a control of the treatment of syphilis. I have tried to include in the work all of the most important data that have been published regarding this very valuable diagnostic measure, and the conclusions that I feel may rightly be drawn from its use in the diagnosis, control of treatment, and as an index of the prevalence of this disease.

The work has been largely prepared since the outbreak of the present war and, for this reason, is not as exhaustive as I had originally intended it to be, as owing to official duties it has been impossible to spend as much time in its preparation as would have been necessary to make it an exhaustive treatise, and it has also been impossible for me to consult much of the very extensive literature that has accumulated during recent years in regard to the test. However, it is believed that the work contains all of the essential and really valuable facts regarding the test which have been reported in the literature, and if there have been any omissions I would deem it a favor to have them called to my attention.

I have quoted quite liberally from some of the more recent investigators, as Noguchi, Nichols, Vedder, and Kolmer, and have also used much data previously published by myself in various medical journals, and it is a pleasure to tender my thanks to the editors of the *Journal of the American Medical Association,* the *Journal of Experimental Medicine,* the *Journal of Infectious Diseases,* and the *American Journal of Syphilis,* for permission to avail myself of the data previously published in these journals.

From personal experience, I believe that there is still a great deal of misunderstanding and confusion among the members of the medical profession regarding the exact value and limitations of the Wassermann test, both in the diagnosis of syphilis, and when used as a control of the treatment of the disease, and if this work will help in clearing up this confusion it will be a source of great gratification. Much of this misunderstanding rests upon the shoulders of laboratory workers, for it must be admitted that too often the performance of the Wassermann test has been delegated to poorly trained or careless assistants, and the results obtained with the test have thus been erroneous and unsatisfactory. I can not urge too strongly upon the profession the necessity for submitting material for this test to well-qualified serologists if reliable results are to be obtained. A standard technic for the test is much to be desired but all efforts in this direction have failed, owing largely to the difficulty of securing a standard antigen, so that at the present time several methods of performing the test are in use, all of which are reliable in the hands of experienced serologists. The method recommended in this work has stood the test of time and has been used by many different workers in thousands of syphilitic infections, and it is believed that it is as simple in technic and as accurate in results as any method of performing the Wassermann test that has been devised.

My thanks are due the publishers for their courtesy and assistance in many ways and for the very excellent manner in which they have reproduced the color plates published in the work.

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CONTENTS

CHAPTER IX

CHAPTER X

BIBLIOGRAPHY

ILLUSTRATIONS

THE WASSERMANN TEST

CHAPTER I

THE DISCOVERY OF THE WASSERMANN TEST

The discovery of the Wassermann test for the diagnosis of syphilis was the direct result of the study of immunity and especially of the discovery of certain facts of basic importance in that process; i. e., bacteriolysis, hemolysis, and complement fixation.

Bacteriolysis

In 1888, Nuttall[1] demonstrated that freshly drawn blood serum and defibrinated blood had a marked destructive effect upon bacteria, an effect that could be definitely estimated, and which was undoubtedly due to some substance or substances in the blood serum and defibrinated blood. This effect consisted in the dissolving of the bacteria and the process was called *bacteriolysis,* the substance causing the destruction of the bacteria being known as a *bacteriolysin.* The bacteriolytic effect of the blood serum or defibrinated blood could be demonstrated in the following manner:

Definite amounts of the blood serum or defibrinated blood were mixed with definite amounts of the bacterium experimented with, and incubated at 37° C. for several hours, after which plates were poured and the number of colonies of bacteria developing compared with the number upon plates containing the same bacterium but to which no blood serum or defibrinated blood had been added. It was ob-

served that the plates prepared from the mixtures of bacteria and blood serum and defibrinated blood showed very many less colonies of the bacterium than those to which no blood serum or defibrinated blood had been added, and, in some of the experiments, the plates were almost sterile.

Nuttall's experiments were confirmed by von Fodor[2] and Buchner,[3] and it was the study and discussion of this subject that introduced into the science of medicine the "humoral" theories regarding the nature of immunity.

Nuttall had shown that the bactericidal power of blood serum was very unstable and that it was only present in fresh blood or blood serum, and that heating the serum at 55 to 56° C., or allowing it to stand for any considerable time destroyed its capacity for dissolving the bacteria. Buchner,[4] in explaining the phenomenon of bacteriolysis, evolved what is now known as the *humoral* explanation, i. e., that the capacity of the blood or serum for dissolving the bacteria resided in a constituent of the fresh blood serum which he termed "alexin;" which he believed to be active against all bacteria; and which he compared to the action of a ferment. Buchner further stated that this "alexin" might be of cellular origin, either a product of the tissue cells or leucocytes. Alexin is identical with the "complement" of Ehrlich and his followers, and in this work the term "complement" will be used in preference to "alexin."

In 1894, Pfeiffer,[5] by his classical experiment upon cholera immunity in the guinea pig, demonstrated that the process of bacteriolysis is a specific one and that the injection of a specific bacterium into an animal is followed by the production of a specific bacteriolysin in the blood serum of that animal for the bacterium injected. His experiment, now a common classroom demonstration of bacteriolysis, was briefly as follows:

If a guinea pig, which has recovered from cholera, be injected in the peritoneal cavity with a suspension containing cholera spirilla, it will be found, upon examination of the

exudate from the peritoneal cavity of the animal, that the spirilla undergo rapid destruction, all stages of the process being visible in samples of the exudate taken at graduated intervals of time after the injection. The spirilla will be observed to become swollen, distorted in shape and granular in appearance, and soon entirely disappear, being dissolved in the peritoneal exudate. That this process is *protective* in nature is shown by the fact that such an animal will survive doses of the spirilla that are lethal to animals that have not been protected by a previous attack of cholera, and that it is *specific* by the fact that other bacteria, when injected into the peritoneal cavity of the cholera pig, are unaffected.

In other words, the experiment proved that the pig, after recovery from cholera, had present in the peritoneal exudate some protective substance, specific in nature, which was capable of dissolving the cholera spirilla and incapable of acting upon other bacteria. This substance was the same as that demonstrated by Nuttall in fresh blood serum and defibrinated blood and to which the name "bacteriolysin" had been given.

A very significant phenomenon observed by Pfeiffer was that if the peritoneal cavity of a normal guinea pig was injected with a mixture of cholera spirilla and some blood serum from the immunized pig, the normal pig also escaped infection. That is, it was possible thus to transfer the bacteriolytic power possessed by the peritoneal exudate of the immunized pig to the peritoneal cavity of an unprotected pig by mixing the cholera spirilla with the blood serum of the protected animal, thus proving that the bacteriolysin also existed in the blood serum. It was also found that while the bactericidal power of the blood serum of the immunized animal was destroyed, *in vitro,* by heating, the heated blood serum is just as effectual in producing immunity in a normal guinea pig when injected with the cholera spirilla as the unheated. This fact Pfeiffer thought to be due to the necessity of some living tissue taking part

in the reaction and stated that the endothelial cells of the peritoneum were probably the cells concerned in this particular experiment. His conclusions, in this respect, lead him to believe that bacteriolysis, so far as it occurred with the cholera spirillum, could only take place in the living body.

The researches of Pfeiffer stimulated a great deal of research upon the subject and to Bordet we owe the true explanation of the process of bacteriolysis. Pfeiffer's conception that it could only occur in the living animal was disproved by Bordet[6] who produced bacteriolysis of the cholera spirillum in hanging drop preparations, and showed, further, that while the bacteriolytic action of immune serum is destroyed by heating the serum at 56° C., the addition to this so-called *"inactivated"* serum of a small amount of fresh normal blood serum, will *"reactivate"* or restore the bacteriolytic property of the serum.

Thus, while normal blood serum may contain no bacteriolytic substances against the organism experimented with, when added to an immune serum which has been heated and its bacteriolytic activity destroyed, it has the power of again bringing the bacteriolytic properties of the immune serum into play and bacteriolysis results. From this experiment it is evident that in the unheated immune serum the dissolving of the bacterium must depend upon *two* substances, one rendered powerless by heat, while the other is unaffected. The substance rendered inactive by heat is said to be *thermolabile,* while the unaffected substance is said to be *thermostable.* It is also evident that the one can not act without the other upon the bacterium, and that the thermolabile body is present in normal, unheated serum, while the thermostable body is only present in the immune serum. Bordet's researches also proved the specific nature of the bacteriolysins and that an animal immunized to several specific bacteria will produce in its blood specific bacteriolysins against the various bacteria injected during the process of immunization.

From the researches mentioned it is evident that the process of *bacteriolysis depends upon the production in the blood of an animal of a specific protective substance which, in the presence of fresh normal blood serum, is capable of dissolving the specific bacterium against which the animal has been immunized. For bacteriolysis to occur there must be present three factors; one, the bacterium used in immunization; the second, the blood serum containing the protective substance produced by immunization; and third, the substance which is present in fresh normal blood serum.*

In immunological literature certain terms have been given the factors mentioned and in this work those used by Ehrlich and his followers will be used. Thus, the bacterium injected in order to produce the bacteriolytic substance is called an *antigen,* and this name may be applied to any substance, which when injected into an animal, results in the formation of an antibody. The bacteriolytic substance, itself, is called an antibody or *amboceptor;* while the substance which is present in fresh normal blood serum and which, when brought into contact with the antigen and amboceptor, causes bacteriolysis is called *complement.* This substance is identical with Buchner's *"alexin."*

Definitions.—As these terms are used, throughout this work, in describing the Wassermann reaction, the following definitions of them are inserted for convenience, as it is essential that they be understood if one desires to have a clear conception of the test:

Antigen.—Any substance which, when injected into suitable animals, will result in the formation of specific antibodies. Antigens may be bacterial, cellular, or chemical in nature.

Amboceptor.—The specific antibody produced by the injection of an antigen. Amboceptors may be normally present in certain blood sera but can be greatly increased by the injection of the suitable antigen. Amboceptors are thermostable, retaining their activity after heating at

56° C. for one half hour. A *bacteriolysin* is a *bacteriolytic amboceptor*. A *hemolysin* is a *hemolytic amboceptor*.

Complement.—The substance present in all fresh normal sera, which when added to a mixture containing antigen and amboceptor, results in the production of bacteriolysis or hemolysis. Complement is rendered inactive by heating for one half hour at 56° C. and is, therefore, thermolabile. It also loses strength rapidly on standing at room temperature.

Bacteriolytic System.—The combination of antigen (bacterium), amboceptor (immune serum), and complement (normal serum) is called a *bacteriolytic* system.

Hemolysis

During his researches upon the nature of bacteriolysis Bordet[7] discovered that if an animal be injected with the red blood corpuscles of another animal a substance is produced in the blood serum of the injected animal capable of laking the red corpuscles injected. To this substance the name *"hemolysin"* is generally applied and the process of laking is known as *hemolysis*. Bordet's discovery of hemolysis was announced in 1898 and at the same time Belfanti and Carbone[8] also described their experiments demonstrating the same phenomenon, so that to these investigators belongs equally the credit for the discovery of one of the most useful and important phenomena of immunity. Although foreshadowed by the work of Landois, who, in 1875, observed that the blood serum of certain animals produced laking of the blood of other species when the two were added together, the real explanation of the process was made clear by the work of Bordet, who proved that, while hemolysins are sometimes present in the freshly drawn blood of certain species against the cells of other species of animals, they can be produced in large amount by the injection of the washed erythrocytes of one species into another, and that the hemolysins thus produced are absolutely specific in nature.

Thus, if a rabbit be injected with the washed red blood corpuscles of man, the hemolysin thus produced in the rabbit's blood serum will cause laking of human erythrocytes but will be powerless against erythrocytes of other species; if injected with the erythrocytes of a sheep, the hemolysin produced will only act upon sheep's erythrocytes; if injected with the erythrocytes of an ox, the rabbit's blood serum will only hemolyse ox corpuscles. In other words, hemolysins are absolutely specific against the kind of erythrocytes injected in immunizing an animal and are powerless against other erythrocytes.

As will be seen, the hemolysins are strictly analogous to the bacteriolysins, being produced by the injection of an antigen, the erythrocytes, the only difference being that in the one case bacteria are dissolved while in the other the hemoglobin is dissolved from the red blood corpuscles employed during immunization. In both instances the reaction will not occur except in the presence of complement, which is furnished by the addition of fresh normal blood serum, and in both instances heating for one half hour at 56° C. destroys the activity of the immune serum, as it destroys the complement present in the serum. Natural hemolysins occur in the blood of one species of animal against the erythrocytes of another, to a limited extent, just as natural bacteriolysins against certain bacteria occur in the blood of certain animals, but these may be greatly increased in amount by the injection of suitable erythrocytes.

To all bacteriolysins and hemolysins the group term "cytolysins" has been applied, owing to the fact that their injurious action is upon the cells injected as antigen in immunization. In the case of the bacteriolysins the bacterium injected constitutes the cell used as antigen, and the injection is followed by the production in the blood serum of a bacteriolysin capable of destroying the bacterium injected; while in the case of the hemolysins, the erythrocyte injected constitutes the antigen and is followed by the production in the animal's blood serum of hemolysins capable

of laking the erythrocytes of the species of animal from which the injected erythrocytes were obtained.

Hemolytic System.—The combination of antigen (erythrocytes), amboceptor (immune serum) and complement (normal serum) is called a *hemolytic system.*

The various theories that have been advanced in explanation of the cause of bacteriolysis and hemolysis and the relative part played in the process by antigen, amboceptor, and complement, will not be discussed here, as it is unnecessary in the consideration of the Wassermann test.

Complement Fixation

In 1906, Bordet and Gengou[9] published their paper describing complement fixation and as the Wassermann test

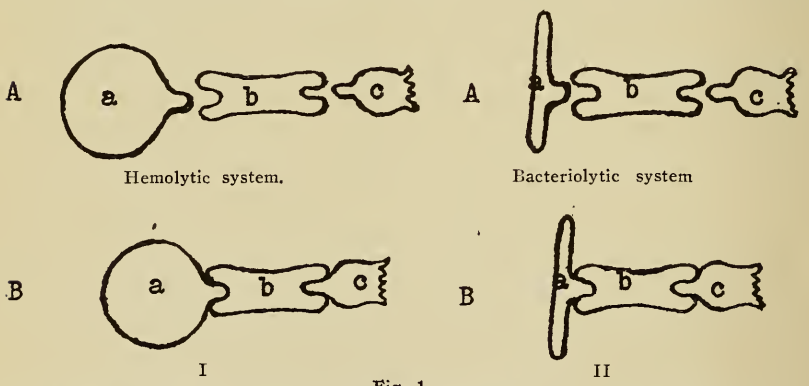

Fig. 1.

I.—*A.* Hemolytic system; *a,* red blood corpuscle, or antigen; *b,* immune rabbit serum, or amboceptor; *c,* guinea pig serum, or complement.
B. Showing union of antigen, *a;* amboceptor, *b;* and complement, *c,* with resulting hemolysis.

II.—*A.* Bacteriolytic system; *a,* bacterium or antigen; b, immune rabbit serum, or amboceptor; *c,* guinea pig serum, or complement.
B. Showing union of antigen, *a;* amboceptor, *b;* and complement, *c,* with resulting bacteriolysis.

is based upon this reaction it is essential that it be thoroughly understood before the technic of the test can be considered.

In experiments upon bacteriolysis and hemolysis it will be remembered that neither process can occur without the presence of three factors; i. e., antigen, amboceptor, and

complement. For instance, if the red corpuscles of a sheep be added to the blood serum of a rabbit which has been immunized to these corpuscles, nothing occurs, but if to this mixture a little fresh normal blood serum be added, the hemoglobin of the red cells is dissolved from them and hemolysis occurs; in the same way, if the erythrocytes and complement, or fresh normal blood serum, be placed together, nothing will occur until the immune serum, or amboceptor be added, when hemolysis results. Furthermore, in neither case is the complement in the normal blood serum fixed by either antigen or amboceptor alone, but can only be fixed, or used up, when both antigen and amboceptor are combined with it. In the reaction the amboceptor, contained in the immune serum, apparently acts as a connecting link, uniting the antigen and the complement and making hemolysis possible.

This fact led Bordet and Gengou to the discovery of complement fixation as a specific test for the differentiation of bacteria, for they found that by using a bacteriolytic and hemolytic system together it is possible to discover the presence of a specific antigen or specific amboceptor in a mixture. In complement-fixation tests the hemolytic system is used as an indicator, as hemolysis is visible to the unaided eye, while bacteriolysis is not.

In order that complement fixation, as applied to the demonstration of specific immune bodies in the blood serum may be understood by the reader, the following hypothetical experiment will be described. The complement-fixation test is not used in the diagnosis of typhoid fever practically, because it is complicated and the agglutination test for the disease is more practical and easier of application, but the experiment described can be carried out in the practice if desired.

Problem: A patient is supposed to be suffering from typhoid fever and it is desired to determine the diagnosis, if possible, by means of complement fixation.

PROCEDURE.—In a test tube containing the necessary amount of normal salt

solution there is placed a certain amount of the patient's serum, which has been inactivated by heating at 56° C. for one half hour; some normal guinea pig serum or complement, and a certain amount of a suspension of typhoid bacilli, or antigen. This mixture is allowed to stand in a water-bath at 37° C. for one-half hour, after which there are added a certain amount of a suspension of human red blood corpuscles and some serum from a rabbit which has been immunized against these erythrocytes. This mixture is allowed to stand in the water-bath at 37° C. for one hour, placed in the ice box for one hour, and the results then noted.

RESULT.—If it is remembered that complement is absolutely necessary in order that hemolysis occur and that complement can not be fixed or used up unless both antigen and amboceptor be present with it, the result of this experiment can be easily understood. If the patient is really suffering from typhoid fever and immune substances or amboceptor be present in the blood serum, the *complement* in the fresh guinea pig serum which is added to the typhoid bacillus suspension, or *antigen,* and the patient's serum which contains *amboceptor* against the typhoid bacillus, will be used up or fixed and bacteriolysis will occur. If now the erythrocytes and the rabbit's serum containing amboceptor against them be added to the mixture, hemolysis will not occur, because the complement has already been used up or fixed by the bacteriolytic system and is no longer available for union with the hemolytic antigen and amboceptor. The test would, therefore, be positive for typhoid, as it would demonstrate that the patient's blood serum contained amboceptor or immune bodies for the typhoid bacillus. On the other hand, if the patient did not have typhoid the complement would not be fixed, so that when the erythrocytes and immune rabbit serum were added, hemolysis would occur, and the test would be called a negative one.

From this experiment it will be seen that the principle of complement fixation can be utilized in the diagnosis of diseases due to a specific microorganism and this fact was quickly recognized and investigators endeavored to work out complement-fixation tests for many of the acute specific infections. Since the discovery of complement fixation the test has been applied to pertussis, gonorrheal infections, tuberculosis, glanders, certain helminthic infections, and several other conditions, but its greatest practical importance was reached in the discovery by Wassermann, Neisser and Bruck of the complement-fixation test for syphilis, or, as it is commonly known, the Wassermann reaction.

Discovery of the Wassermann Complement-Fixation Test for Syphilis.—Many students of the subject, after the dis-

covery of complement fixation by Bordet and Gengou, were able to show that it was not necessary to employ the whole bacteria as antigen in making tests but that watery extracts of the bacteria, when used as antigen, gave equally good results. This fact caused Wassermann and Bruck to conclude that tuberculins should act well as antigen in a complement-fixation test for tuberculosis, and it was while working upon this problem that Wassermann conceived the idea that complement fixation could be applied to the diagnosis of syphilis. The discovery of *Treponema pallidum,* by Schaudinn, had shown that the disease was due to an organism that is present in large numbers in the tissues of certain organs, and to these organs Wassermann turned for his antigenic extract, as *Treponema pallidum* had not then been grown in pure culture.

Wassermann, working in conjunction with Neisser and Bruck,[10] attempted to produce complement fixation in syphilis by using the blood serum of syphilitic monkeys as amboceptor and watery extracts of syphilitic organs as antigen, and, as expected, it was found that such a combination resulted in complement fixation. It was but a step from this experiment to the application of the test to man, and in 1906, Wassermann, Neisser, Bruck, and Schucht[11] published a paper giving their results in the diagnosis of syphilis in man by the complement-fixation test. Their paper was antedated a short time by that of Detre,[12] but as Wassermann, Neisser and Bruck, in their previous publication describing the test upon syphilitic monkeys antedated Detre's paper, the credit for the discovery remains with Wassermann and his colleagues.

Wassermann considered the complement-fixation reaction in syphilis as a true antigen-antibody reaction, the fixation being due to specific antibodies or amboceptors present in the patient's blood, produced by *Treponema pallidum,* the etiological agent of the disease. This conception of the reaction was held by all observers until 1907, when several investigators demonstrated that complement fixa-

tion in syphilis occurred not only when an extract of tissues containing *Treponema pallidum* was used as antigen, but also when nonspecific substances were used, as extracts of normal tissues and even certain chemical reagents. In 1907, Weygandt[13] obtained a positive reaction in tabes by using an antigen obtained by extracting a normal spleen, and Marie and Levaditi[14] obtained positive reactions in paresis with an aqueous extract of normal liver. Later, Plaut,[15] Landsteiner,[16] and others obtained positive results in complement fixation in syphilis by using aqueous extracts of normal organs, both of man and animals, as antigen, and finally, the publication of the paper by Landsteiner, Müller and Pötzl,[17] in which they demonstrated that as good results could be obtained in the diagnosis of syphilis by the complement-fixation test with alcoholic extracts of guinea pig heart as antigen as when aqueous extracts of foetal syphilitic liver were employed, demonstrated that the reaction could no longer be considered specific in the sense that it is a true antigen-antibody reaction.

The work of the last named investigators was soon confirmed by others and it was soon shown that alcoholic extracts of tissues were as valuable as antigens in the Wassermann test as watery extracts, and that normal organs of man or animals were apparently as rich in complement-fixing substances as were organs containing *Treponema pallidum.*

Noguchi[18] added greatly to our knowledge of the subject by proving that the substances which fix complement in the Wassermann reaction are acetone insoluble, thus indicating that they consisted largely of lipoids, and a further analysis showed that such antigenic extracts contained lecithin, cholesterin, and sodium oleate. The fact that the addition of a certain amount of cholesterin to antigens used in the diagnosis of syphilis greatly increased their antigenic value was first noted by Browning and Cruickshank[19] and was soon confirmed by other workers, notably, in this coun-

try, by Walker and Swift,[20] and at the present time such cholesterinized antigens are very generally used and, in the experience of the writer and most authorities who have used them, are perfectly reliable when properly controlled and are more valuable in the specific diagnosis of syphilis by the Wassermann test than are antigens which have not been thus fortified.

The discovery that the antigens used in the Wassermann test need not be prepared from tissues containing *Treponema pallidum* but that alcoholic extracts of normal tissues are just as effective, and that certain chemicals, as cholesterin, when added to antigens prepared from normal tissues, increase their antigenic value in the diagnosis of the disease, demonstrated, beyond question, that the early conception of Wassermann that complement fixation in syphilis is a specific antigen-antibody reaction, is erroneous, and that this reaction can no longer be considered a specific one on the strict sense in which this term is employed in immunology. Although this is undoubtedly true, the practical application of the test in many hundreds of thousands of cases of syphilis has proved that, except in a very few easily recognized conditions, the test *is specific* for syphilis and that, clinically, it is probably even more valuable than it would be if it were a true antigen-antibody reaction, for it is doubtful if as large a percentage of infections would react positively to the test if this were true, judging from the results of complement fixation in other diseases which are true antigen-antibody reactions.

Although, as generally performed, the Wassermann test is not a true specific reaction, the work of Noguchi[21] and of Nichols and the writer[22] has proven that, with antigens prepared from pure cultures of *Treponema pallidum* complement fixation can be obtained with syphilitic sera, and that in such instances the reaction is really a specific one, due to antibodies in the patient's blood serum against the treponema. This subject will be discussed in a later chapter. (Chapter VI.)

CHAPTER II

GENERAL DESCRIPTION OF THE WASSERMANN TEST. NATURE OF THE REACTION

As stated in the preceding chapter, Wassermann and his colleagues considered the complement-fixation test for syphilis as a true antigen-antibody reaction, the aqueous extract of tissue containing *Treponema pallidum* furnishing the specific antigen, while the patient's blood serum contained the specific antibody or amboceptor. This conception of the reaction had to be abandoned when it was demonstrated that aqueous or alcoholic extracts of normal tissues could serve equally well as an antigen in the test. Therefore, it follows that the terms "antigen" and "amboceptor" when used in reference to this test, except when applied to the hemolytic system employed, are not strictly accurate, inasmuch as the antigenic extracts are not true antigens, their injection into animals not being followed by the development of antibodies; while the substance or substances in the syphilitic patient's serum which, in the presence of complement and the antigenic extract, fix the former, are not true amboceptors, for they are not produced by injection of the antigenic extracts employed in the test. However, as the reaction agrees in all essentials with really specific complement-fixation reactions, and as the antigenic extracts and the syphilitic patient's serum act in the same manner as do true antigens and antibodies, it is convenient to retain these terms in speaking of the reaction.

As already stated the discovery of complement fixation by Bordet and Gengou was quickly utilized in the diagnosis of infectious diseases and the Wassermann test is simply an adaptation of the principle to the diagnosis of syphilis. In performing the test a hemolytic system is used as an

indicator and what corresponds with a bacteriolytic system is used as the diagnostic system. The hemolytic system consists of a suspension of erythrocytes, the antigen; an immune serum containing antibodies to the erythrocytes used as antigen, the amboceptor; and fresh normal blood serum, the complement. The bacteriolytic system of true specific complement-fixation reactions is replaced by the syphilitic system, as it may be called, consisting of an extract of syphilitic or normal tissues, the antigen; the syphilitic patient's serum, corresponding to the amboceptor; and complement, or fresh normal blood serum.

These various reagents are employed in exactly the same manner as are the hemolytic and bacteriolytic systems in specific complement-fixation reactions, as will be apparent from the general description of the test which follows:

It will be remembered that in neither a hemolytic nor bacteriolytic experiment will either hemolysis or bacteriolysis be brought about unless all three factors necessary for this to occur are brought together; i. e., antigen, amboceptor and complement must be placed in the same mixture before either hemolysis or bacteriolysis can occur. The immune serum contains the amboceptor and this body acts as an intermediary or connecting link between the antigen and complement, and when this union occurs either hemolysis or bacteriolysis results, as the case may be. In the Wassermann test the patient's blood serum, if he is syphilitic, acts as the amboceptor, the serum containing some substance or substances capable of uniting with antigen (the extract employed) and complement and binding the latter. In order to demonstrate the presence of this complement binding substance in the patient's serum the following procedure is employed, and it is this procedure that is known as the complement-fixation test for syphilis or the Wassermann test:

In a test tube containing a suitable amount of normal salt solution there is placed a certain amount of the blood serum from the patient to be tested; a certain amount of

fresh normal blood serum, generally from a guinea pig, which contains complement; and a certain amount of an extract of syphilitic or normal tissues, the antigen. This mixture is allowed to stand in a water-bath at 37° C. for one half hour, at the end of which time there are added to the

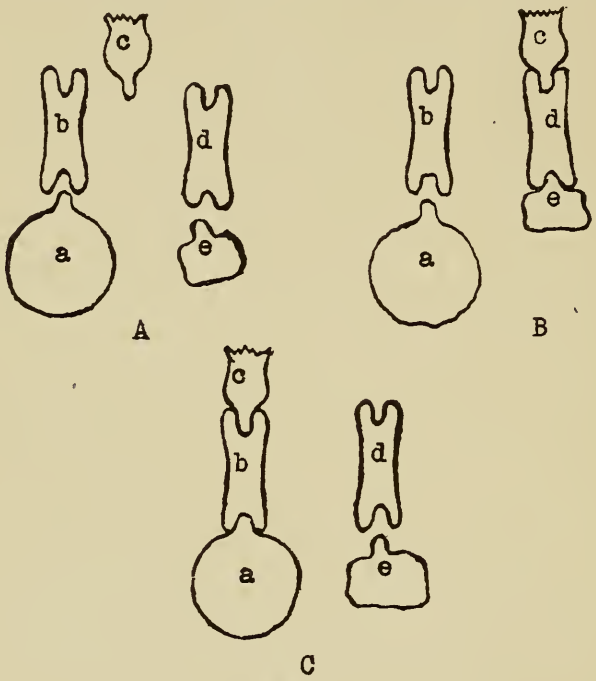

Fig. 2.

Schematic representation of complement fixation in syphilis or the Wassermann test.

A. The reagents entering into the reaction; *a*, human red blood corpuscles or hemolytic antigen; *b*, hemolytic amboceptor, or immune rabbit serum; *c*, complement, or guinea pig serum; *d*, patient's blood serum, which may, or may not contain syphilitic antibody; *e*, syphilitic antigen or antigenic extract.

B. Representing a *positive* reaction. The patient's blood serum, *d*, containing syphilitic amboceptor, has caused the union of complement, *c*, and syphilitic antigen, *e*, so that when the hemolytic antigen, *a*, and the hemolytic amboceptor, *b*, are added to the mixture, no hemolysis results, as the complement has already been fixed or absorbed, as shown in the figure.

C. Representing a *negative* reaction. The patient's blood serum, *d*, containing no syphilitic amboceptor, does not fix the complement with the syphilitic antigen, *e*, so that when the hemolytic antigen, *a*, and the hemolytic amboceptor, *b*, are added, union occurs between them and the complement, and hemolysis results.

mixture a certain amount of a suspension of human or other red blood corpuscles and a certain amount of the blood serum of a rabbit immunized to the blood corpuscles

employed in the suspension. The mixture is again incubated in a water-bath at 37° C., this time for one hour, and then placed in an ice box, and the result noted at the end of an hour or two.

If the patient is suffering from syphilis, the result will be that no hemolysis occurs, and the test is called positive, while, if syphilis is not present, hemolysis will occur, and the test is said to be negative. The *positive* result is due to the fact that during the first incubation the substance in the patient's blood serum, corresponding to a specific amboceptor, unites the antigen, or extract used in the test and the complement, thus binding the latter, so that when later the erythrocytes and immune serum or amboceptor against them is added, there is no complement left to unite with them and therefore, hemolysis can not occur. In case that the result is *negative,* this is due to the fact that the patient's blood serum contained no substance capable of uniting the antigenic extract and complement, so that when later the erythrocytes and the immune serum to them were added, the amboceptor in the immune serum caused the union of the erythrocytes and the complement and hemolysis resulted, the complement having been left free in the mixture, owing to nonunion during the first incubation.

From this brief description of the Wassermann test it will be noted that it corresponds in every way as regards technic and results with specific complement-fixation tests, but that instead of a specific bacteriolytic antigen there is substituted a nonspecific tissue extract which serves as an antigen and which acts in the same manner as does the specific antigen employed in bacteriolytic experiments, while the complement-fixing substance or substances in the syphilitic patient's blood serum act in the same manner as does the amboceptor in the immune serum in bacteriolytic experiments. When, however, an extract of a pure culture of *Treponema pallidum* is used as an antigen there is no difference between the Wassermann test and other com-

plement fixation reactions, as in this case the complement fixation is the result of a true antigen-antibody reaction. Unfortunately, the antigens prepared from pure cultures of *Treponema pallidum* are very unreliable in practice, so that they can not be used for this purpose, and the nonspecific antigens have to be depended upon in the test.

Nature of the Wassermann Reaction

The exact nature of the Wassermann reaction is still a mystery, despite an immense amount of work that has been done in the endeavor to solve the problem. The fact that extracts of tissues which do not contain *Treponema pallidum* serve equally well as antigens as those that do, and even better than antigens prepared from pure cultures of the organism, proves that the reaction is not a true antigen-antibody reaction. The experiments of Noguchi[23] demonstrating that the active antigenic principles in the normal tissue extracts were lipoids and his conclusion that the reaction depends upon lipotropic substances in the patient's blood serum, showed that the cause of the reaction is closely connected with the chemistry of the lipoids, and this is about all that we can claim to know regarding its exact nature to date, although many ingenious and interesting theories have been advanced in explanation of the phenomenon.

Citron[24] adopted the hypothesis that the antibody producing antigen is a toxolipoid and has proposed the name "Luesargine" for the syphilitic antibodies. In conjunction with Münck he claims to have demonstrated that the true syphilitic antigen is contained only in aqueous extracts of syphilitic tissues and that only rabbits inoculated with such extracts produce antibodies that react positively with alcoholic extracts of normal organs, but his work has not been confirmed.

Schmidt[25] considers that the reaction is colloidal in nature, depending upon the union of the colloids in the anti-

genic extracts and the serum globulins in the patient's blood serum, which are increased in amount and changed in nature in syphilitic patients.

Levaditi and Yamanouchi[26] believe that the reaction depends upon the union of two colloidal factors, one present in the patient's blood serum and nonproteid in character, the other consisting of lipoidal substances in the extracts used as antigen.

Weil and Braun[27] conclude that during the progress of the disease certain tissue products, lipoidal in nature, are absorbed, and give rise to antibodies which will react *in vitro* with the lipoids present in the antigenic extracts, the so-called autoantibody theory. This theory is somewhat similar to Citron's, both resting upon the belief that in the tissues of the patient there are produced antibodies to the lipoids in the antigenic extracts, these antibodies being produced by the absorption in the body of albumin-lipoid substances produced by the growth of *Treponema pallidum,* and the subsequent production of antibodies against these substances.

Bergel[28] considers that the Wassermann reaction may be due to the action of a specific lipase which is produced in the syphilitic patient against "lues-lipoids." This theory can not hold in the light of the facts demonstrating that the reaction occurs with extracts prepared from normal organs, when used as antigen.

Manwaring[29] thinks that in guinea pig blood serum there is a proteolytic ferment which may weaken or destroy complement and that the fixation reaction is due to substances present both in the antigenic extracts and syphilitic patient's serum which increase this effect. Bruck and Stern[30] believe that the reaction is due to the interaction of the lipoid bodies in the antigenic extracts and similar bodies in the patient's blood serum; while Peritz[31] concludes that it is due to some substance in the blood serum that has an affinity for lecithin, which is always contained in the extracts.

None of the various theories advanced to account for this reaction have been accepted as the true explanation of the process of complement fixation in syphilis and none of them rest upon sufficient experimental evidence to be accepted as the explanation. The most that can be said regarding the reaction is that it is due to an interaction between lipotropic bodies present in the blood serum of syphilitics and lipoids in the antigenic extracts. A mixture of a lipoid extract and syphilitic blood will absorb a large amount of complement, so that it follows that the substance or substances in the serum must be lipotropic in nature. This lipotropic substance is more constantly present in the blood of syphilitics than in other diseases, and is practically specific for that disease. Researches have conclusively proved that the lipotropic substance or substances are due to unknown changes in the tissues or blood serum brought about by the actual presence in the tissues of living *Treponema pallidum,* and that the reaction disappears with the cure of the infection, so that its presence is proof of the existence of syphilis even though months or years may have passed without definite clinical symptoms having been observed.

Although the Wassermann reaction, as the test is usually performed, is not a true antigen-antibody reaction, there is proof that in the blood of syphilitics true antibodies against *Treponema pallidum* are formed which will fix complement in the presence of antigens prepared from pure cultures of the organism.

The first experiments along this line were initiated by Schereschewsky,[32] who obtained positive reactions in syphilitics by using antigens made from an impure culture of what he regarded as *Treponema pallidum.* Owing to the doubt attending the impurity of the culture and as to the exact species of treponema with which he worked, Schereschewsky's results were not conclusive, and it was not until Noguchi[33] cultivated in pure culture the organism of syphilis and employed antigens prepared from these pure cul-

tures, that it was proved that true complement fixation did occur in syphilis. Noguchi[34] demonstrated, that by using aqueous extracts of pure cultures of *Treponema pallidum* as antigen, it was possible to secure complement fixation in certain cases of the disease, but that the reaction did not occur as consistently as when nonspecific antigens were employed nor did it occur at all stages of the disease. Noguchi immunized rabbits with the pure cultures, using the aqueous pallidum extracts for injection, and found that their blood bound complement when the pallidum extract was used as antigen but that negative results were obtained when extracts of normal tissues were used as antigens. These results proved that the blood serum of the immunized rabbits contained a real antibody against the syphilitic toxin contained in the pallidum extract used in immunization. He found, however, that while the blood serum of rabbits with an active syphilitic orchitis, gave positive complement fixation with the extracts of normal tissue, rich in lipoids, it did not bind complement when the pallidum extract was used. He explained this result as follows:[35]

> The findings show that the immune serums contain the specific antibodies for the pallidum but not for the lipoids, and that in the serums of syphilitic rabbits (active stage) there is an abundance of the lipotropic substance (probably an enzyme) capable of rendering the complement inactive (so-called fixation) in the presence of certain lipoids, but too little specific antibodies to bind complement with the pallidum antigen.

When pure pallidum antigens were used with human syphilitic blood serum, Noguchi obtained similar results, the early stages of the disease, primary and secondary, seldom reacting with the specific antigen, while in the tertiary, latent, and late congenital cases the blood serum frequently gave a positive result. Because of these diverse results in the various stages of the disease, when the pure pallidum antigen was used, Noguchi concluded that a positive result with the pallidum antigen indicated the formation of true antibodies in the blood of the patient, and was,

therefore, of favorable omen, while a negative reaction indicated their absence.

In 1912, the writer and Nichols,[36] using antigens made by extracting pure cultures of *Treponema pallidum* with alcohol, obtained complement fixation in all stages of syphilis, but positive reactions were also obtained with syphilitic blood serum with antigens made from pure cultures of *Treponema pertenue, Treponema microdentium,* and *Spirocheta refringens,* while antigens made from pure cultures of organisms like *Bacillus typhosus* and *Spirillum choleræ* gave negative results. We concluded that the positive reactions with the antigens prepared from the organisms mentioned was in the nature of a group reaction, and that the practical value of the test when the pallidum antigen was used was small, owing to the fact that many cases of the disease gave a negative reaction, even during the late stages of the infection. Our results have since been confirmed by Kolmer, Williams and Laubaugh[37] and other investigators.

While it may be considered as proved that antigens prepared from pure cultures of *Treponema pallidum* are unreliable and of little value in the practical application of the Wassermann test, their use has demonstrated that a specific fixation of complement may be obtained in syphilis when such antigens are used in the complement-fixation test. This fact renders it very probable that antigens prepared from foetal syphilitic liver, rich in treponemas, may give positive results in rare instances where extracts of normal tissues will fail, and the use of the two kinds of antigen in practice has shown that this is the case. The writer is, therefore, of the opinion that the Wassermann reaction, when an extract of foetal syphilitic liver is used as antigen, is of a dual nature, in some instances, consisting of a true antigen-antibody reaction between specific antibodies in the patient's blood serum and specific substances in the antigen; and a nonspecific reaction between the lipotropic substance in the patient's serum and lipoids

in the antigen. When an extract of normal tissues is used as antigen the reaction is due to the lipoids in the antigen and lipotropic substances in the patient's blood serum, and, in such instances, the possible reaction between true antibodies and the specific substance in the antigen is lost, and the reaction may be negative instead of positive. While, in practice, in the writer's experience, this result very rarely occurs, it is considered best, if possible, to make use of both varieties of antigen when performing the Wassermann test.

CHAPTER III

FACTORS WHICH INFLUENCE THE RESULT OF THE TEST

The Wassermann test is quantitative in nature and unless all of the reagents are carefully titrated at frequent intervals and accurately used, erroneous results are sure to occur. The quantity of blood serum to be tested; its proper inactivation; the dose of complement and its source; and the amount of amboceptor and antigen to be used; are all important factors in the test and variations from the proper amount of each will be followed by faulty results, as will be shown in the chapter devoted to technic.

However, aside from the reagents employed in the test there are certain factors which have a very marked influence upon its result and which should be understood by physicians using this valuable diagnostic measure. These factors are: The influence of the ingestion of alcohol; the influence of certain bacteria in the blood serum; and variations in the amount of complement inhibiting substance in the blood serum to be tested.

The Influence of the Ingestion of Alcohol.—In 1911, Major Nichols, of the Medical Corps of the Army, and the writer[38] called attention to the effect that the ingestion of considerable amounts of alcohol has upon the results of the Wassermann test. It was found that the ingestion of alcohol in the form of beer or whiskey, and in considerable amounts, might convert a strongly positive serum into a negative one, if the test were made within twenty-four hours after the ingestion of the alcohol, and that the blood might remain negative for as long as three days, although it generally became positive again within twenty-four hours. This fact was discovered by the writer

while making routine examinations of the blood of a certain individual who was being treated for syphilis by Major Nichols, and who had presented several strong positive reactions before the first negative one which was due to the ingestion of alcohol, and further research by Nichols and myself proved that this drug has a very potent influence in weakening or destroying the lipotropic substances in the patient's blood serum which apparently cause the Wassermann reaction.

The case in which this phenomenon was first observed is of special interest in this connection and the clinical history is here given:

Male, 41 years of age. Infected with syphilis in 1904. Now has palmar lesions. February 25, 1911, 0.5 gm. of salvarsan was administered intravenously, the Wassermann test before administration giving a plus reaction. Between this date and April 7th, 1911, his blood was tested five times, the reaction four times being double-plus, and the last test, on April 7th, being plus. (Double-plus here equals four-plus of most authorities.) April 8th his blood was again tested and was found to be negative. The result was unusual and on inquiry it was learned that on the night of April 7th he drank about 700 c.c. of Munich beer. His blood was tested again on April 12th, and gave a double-plus (four-plus of some authors) reaction. Believing that the beer might have had something to do with the sudden disappearance of the positive reaction in this case, we requested that he repeat the beer on the night of April 14th, which he did, and on the morning of April 15th the reaction was again negative. On the 17th of April it had become plus and on the 22nd again was double-plus.

As it was evident that the negative result of the Wassermann test was due, in this case, to the ingestion of the beer, we decided to repeat the experiments on other cases. We selected nine syphilitic patients, who before the administration of alcohol gave a double-plus reaction, and Table I gives the results obtained in these patients. It will be remembered that in this table, the double-plus reaction means absolute inhibition of hemolysis, and is equivalent to the four-plus reaction of most writers.

Of the 9 cases included in this table, 3 were in the secondary stage of the disease and 6 were in the tertiary stage. The quantity of alcohol administered varies some-

TABLE I

ILLUSTRATING THE EFFECT OF THE INGESTION OF ALCOHOL UPON THE RESULT OF THE WASSERMANN TEST

CASE NO.	STAGE OF DISEASE	*Before alcohol*		*Alcohol given*		*Date and result after alcohol*		
		REACTION	DATE 1911	DATE 1911	AMOUNT	DATE 1911	TIME	RESULT
1		1st test, +	Apr. 1	Apr. 7	700 c.c. Munich beer in evening	Apr. 8	9 A.M.	−
						Apr. 12	9 A.M.	++
		2nd test, ++	Apr. 12	Apr. 14	Same amount of beer, same time	Apr. 15	9 A.M.	−
						Apr. 17	9 A.M.	+
						Apr. 22	9 A.M.	++
2	2nd	++	May 19	May 20	90 c.c. of 95% alcohol, 7 A.M. to 7 P.M.	May 20	7:30 P.M.	+
						May 21	10 A.M.	−
						May 22	10 A.M.	+−
						May 23	10 A.M.	++
						May 24	10 A.M.	++
3	3rd	++ one hour before alcohol	May 29	May 29	240 c.c. whiskey between 10 A.M. and 5 P.M.	May 29	6 P.M.	+
						May 30	6 A.M.	−
						May 31	10 A.M.	+−
						June 1	10 A.M.	++
						June 2	10 A.M.	++
4	3rd	++ one hour before alcohol	June 3	June 3	240 c.c. whiskey between 10 A.M. and 6 P.M.	June 3	8 P.M.	−
						June 4	7 A.M.	−
						June 5	10 A.M.	+
						June 6	10 A.M.	++
5	2nd	++ day before alcohol	June 4	June 5	180 c.c. whiskey between 9 A.M. and 6 P.M.	June 5	8 P.M.	−
						June 6	8 A.M.	−
						June 7	8 A.M.	++
						June 8	8 A.M.	++
6	2nd	++ one hour before alcohol	June 22	June 22	240 c.c. whiskey between 9 A.M. and 7 P.M.	June 23	9 A.M.	−
						June 23	8 P.M.	−
						June 24	9 A.M.	+−
						June 25	9 A.M.	++
7	3rd	++ one hour before alcohol	June 29	June 29	240 c.c. whiskey between 10 A.M. and 5 P.M.	June 26	6 P.M.	++
						June 30	6 A.M.	−
						July 1	6 A.M.	+
						July 2	6 A.M.	++
8	3rd	++ one hour before alcohol	July 7	July 7	240 c.c. whiskey between 12 M. and 5 P.M.	July 7	8 P.M.	−
						July 8	1 P.M.	−
						July 9	1 P.M.	−
9	3rd	++ one hour before alcohol	July 7	July 7	240 c.c. whiskey between 12 M. and 7 P.M.	July 8	1 P.M.	++
						July 9	1 P.M.	−
						July 10	1 P.M.	−
						July 11	1 P.M.	+−
						July 12	1 P.M.	+
						July 13	1 P.M.	++

what but in every case the positive reaction disappeared after its administration. In most of the cases the blood was tested one hour before the administration of the alcohol was begun as well as afterward. A study of the table shows that in 5 of the cases the reaction was found to be negative one hour after the last dose of the alcoholic liquor had been administered, and that in all the cases the reaction remained negative for several hours. In cases 4, 5, and 6 the reaction remained negative for over 24 hours while in case 9 it remained negative for three days and did not become double-plus again until over four days had elapsed from the time the alcohol was administered. Two of the patients had received salvarsan before the administration of the alcohol, but in one the drug had been given so long before that it could not have had any effect upon the reaction, while in the other the fact that the reaction became double-plus again after becoming negative from the alcohol, proves that the salvarsan had nothing to do with its disappearance.

That the disappearance of the reaction after the ingestion of alcohol is not due to the alcohol itself, which may be present in the blood serum, is proved by the fact that a much larger quantity of alcohol is necessary to produce hemolysis, as proved by experiment, than could possibly be present in the blood serum of the patients tested. Thus, we found that it requires at least 0.15 c.c. of absolute alcohol to produce complete hemolysis of 1 c.c. of a 1 per cent suspension of human red blood corpuscles within the time used in the Wassermann test for incubation of the hemolytic system, whereas only 0.08 c.c. of the patient's blood serum was used in each test. It is thus evident that if the amount of blood serum used had been actually absolute alcohol the quantity would have been insufficient to have produced hemolysis.

From these experiments, which have been confirmed by many investigators since our original publication, it is evident that alcohol may render inert the sub-

stance or substances in the blood serum of syphilitics which react with lipoids in the antigenic extracts, and thus a strongly positive serum may give a negative result. For this to occur, the alcohol must be taken in considerable quantity and probably within twenty-four hours, or at most three days before the test is made, but it should be remembered that smaller amounts of alcohol may render weak reactions negative, so that cases which should present a single plus reaction will often react negatively after even moderate amounts of alcoholic liquors have been ingested. It is thus evident that very careful inquiry should be made regarding the use of alcoholic liquors before taking blood for a Wassermann test from any patient, and if there is a history of indulgence in such liquors within from twenty-four to thirty-six hours previously, the taking of the specimen should be postponed and alcoholics forbidden until at least two days have passed. The neglect of this precaution has often resulted in erroneous results and cases that should have been strongly positive have been reported as negative or doubtful. When the test is used as a control of treatment alcohol, even in small amounts, should be forbidden for some days before the blood is collected, or the weak reactions often observed in treated cases will be entirely missed, thus obscuring the real condition of the patient as regards his serological status.

From what has been said, it follows that a negative Wassermann reaction, after the ingestion of alcoholic liquors, possesses no value whatever, and an immense amount of harm has doubtless been done by lack of care in ascertaining whether patients have indulged in such liquors within a short time before the blood was collected. Our observations, as well as those of others, both in this country and abroad, have proved that the effect of alcohol upon the Wassermann reaction is very important from a practical standpoint, and one which should not be forgotten when

patients come to the physician for the collection of blood for the test.

The Influence of Certain Bacteria, when Developing in the Blood Serum, upon the Results of the Test.—It is a well-known fact that, upon standing for varying lengths of time, some human blood sera develop anticomplementary substances, which cause inhibition of hemolysis in both the tubes containing antigen and the control tubes used in the Wassermann test. Most of these bodies are destroyed by heating the blood serum for one-half hour at 56° C., but, in some instances, they still persist after heating, and in such sera one is unable to read the result of the test owing to inhibition occurring in both the antigen and control tubes. From these observations it is evident that these anticomplementary bodies are of two kinds, thermolabile and thermostable, and that the inactivation of the blood serum by heating enables us to destroy only the thermolabile group.

While the exact nature of these anticomplementary bodies is still doubtful, the writer has shown[39] that some of them are produced by the growth of bacteria in the blood serum to be tested, if the serum is kept for some time. Not only will certain bacteria produce anticomplementary bodies that cause inhibition of hemolysis in both the antigen and control tubes of the test, but certain species also possess the power of causing inhibition of hemolysis in the antigen tube alone, thus giving rise to a nonspecific reaction in the sera containing them. It is these species that are of special importance in the application of the Wassermann test and which will here be discussed.

While carrying on some comparative tests of blood serum with another laboratory it was noted that in one instance which gave a positive reaction in the writer's laboratory and a negative one at another laboratory situated five days' journey distant, the blood serum when received was cloudy in appearance and had a bad odor. As it was known to be infected and as it was thought possible that

bodies developed in the serum by the growth of the bacteria might be the cause of the difference in results, this serum was plated out upon agar-agar and cultures of the following organisms were isolated: *Staphylococcus aureus;* a large *Staphylococcus albus;* a small *Staphylococcus albus,* a large spore-bearing bacillus of the *subtilis* type; and a very minute diplobacillus.

In order to determine whether any of these bacteria were responsible for the reaction obtained with this blood serum the following experiments were undertaken together with experiments with a stock culture of *Staphylococcus aureus, Staphylococcus citreus,* and *Streptococcus pyogenes.* The same technic was followed in these experiments as was used in performing the Wassermann test, the mixtures of serum and bacteria being substituted for the patient's blood serum.

Experiment 1.—An emulsion in normal salt solution was made from a pure culture of each organism isolated from the blood serum, and 0.08 c.c. of this emulsion was placed in 1 c.c. of a 1 per cent suspension of human red blood corpuscles, together with 0.08 c.c. of an inactivated normal serum. Table II gives the result of this experiment.

TABLE II

RESULTS OF COMPLEMENT-FIXATION TESTS IN EXPERIMENT NO. 1

NAME OF BACTERIUM	*Results*		*Controls*		ORIGIN OF BACTERIUM
	ANTIGEN TUBE	CONTROL TUBE	BACTERIA ALONE	SERUM ALONE	
S. albus, large	–	–	–	–	Isolated
S. albus, small	–	–	–	–	from
S. aureus,	–	–	–	–	serum in
Bacillus (Spore)	–	–	–	–	all cases
Diplobacillus	–	–	–	–	
Combined bacteria	–	–	–	–	

From these results it is evident that merely adding an emulsion of the cultures of these bacteria to normal blood serum is without effect upon the result of the complement-fixation test, as all of the mixtures gave a negative reaction.

Experiment 2.—This experiment was identical with Experiment 1, except that the mixtures of serum and bacteria were inactivated at 56° C. for one-half

hour before being tested. The mixtures of bacteria and serum in this experiment all gave a negative reaction with the exception of the mixture of blood serum and the diplobacillus, which resulted in a plus reaction, the heating resulting in the liberation of enough inhibitory substances to cause this type of reaction in both the antigen and control tubes. Of course this reaction would have caused no confusion so far as the diagnosis of syphilis is concerned, as the fact that the inhibition occurred in the control tube as well as in the antigen tube would have thrown the test out entirely and another specimen would have been requested.

Experiment 3.—Specimens of normal serum were inoculated with each of the bacteria isolated from the serum in question as well as with the stock cultures and allowed to stand at room temperature for one week before being tested. Table III gives the results of this experiment:

TABLE III

RESULTS OF COMPLEMENT-FIXATION TESTS IN EXPERIMENT NO. 3

NAME OF BACTERIUM	*Result*		CONTROL, SERUM ALONE	ORIGIN OF BACTERIUM
	ANTIGEN TUBE	CONTROL TUBE		
S. albus, large	+–	–	–	Isolated from serum
S. albus, small	–	–	–	" " "
S. aureus,	–	–	–	" " "
Bacillus (spore)	–	–	–	" " "
Diplobacillus	+	++	–	" " "
S. aureus	++	–	–	Stock culture
S. citreus	++	++	–	" "
S. pyogenes	–	–	–	" "

It is evident, by consulting the table, that after incubation in human blood serum for a week at room temperature the large *S. albus* isolated from the blood serum under discussion gave a plus-minus reaction in the antigen tube, while the diplobacillus isolated from the same serum gave a plus reaction in the antigen tube and a double-plus reaction in the control tube. The stock *S. aureus* gave a double-plus reaction in the antigen tube, while the stock culture of *S. citreus* caused total inhibition of hemolysis in both antigen and control tubes.

From this experiment it is evident that the stock *S. aureus*, growing in normal blood serum at room temperature for one week produced some substance or substances in the serum which gave a false positive reaction.

Experiment 4.—This experiment was identical with Experiment 3, except that the mixtures of bacteria and serum were incubated at 37° C. for 24 hours

and then allowed to stand at room temperature for one week, before being tested. Table IV gives the results of this experiment:

TABLE IV

RESULTS OF COMPLEMENT-FIXATION TESTS IN EXPERIMENT NO. 4

NAME OF BACTERIUM	*Result*		CONTROL SERUM ALONE	ORIGIN OF BACTERIUM
	ANTIGEN TUBE	CONTROL TUBE		
S. albus, large	++	–	–	Isolated from serum
S. albus, small	–	–	–	" " "
S. aureus	++	–	–	" " "
Bacillus (spore)	–	–	–	" " "
Diplobacillus	++	++	–	" " "
S. aureus	++	–	–	Stock culture
S. citreus	++	++	–	" "
S. pyogenes	++	–	–	" "

From this experimental record it will be noted that no less than four of the organisms tested gave a double-plus (four-plus) positive reaction in the antigen tube alone, while two inhibited hemolysis completely in both the antigen and control tubes. It would appear that the 24 hours at incubator temperature lead to an increase in the intensity of the reaction, due to a richer development of the bacteria in the sera.

Considering the experiments as a whole it is perfectly evident that a positive complement-fixation reaction may be obtained in a normal serum if certain species of bacteria are enabled to develop within it. That every strain of a bacterial species will not produce this result is shown by the fact that while a double-plus reaction was obtained in the serum infected with the stock *S. aureus* and with the *S. aureus* isolated from the serum under discussion, a negative result was later obtained with an *S. aureus* isolated from a blood serum that gave a negative reaction.

It is also evident that temperature has much to do with the ability of the bacteria to produce the positive reaction, for in the mixtures of serum and bacteria kept for 24 hours at incubator temperature, the reactions were most marked, while two of the organisms which did not give a reaction when growing in the blood serum at room tem-

perature, gave a strongly positive reaction when incubated. Inactivation also appears to favor the production of the false positive reaction in sera which are contaminated with certain bacteria.

As regards the serum under discussion, it is evident that the positive result could have been caused by either the large *S. albus* or the *S. aureus,* or both, according as the conditions at the time of examination were favorable to the production of inhibitory substances by one or both of these organisms.

The fact that, under certain conditions, such common bacteria as staphylococci and streptococci, when growing in normal blood serum, may give rise to a positive Wassermann reaction, is of great practical importance, and one might conclude from these experiments that only fresh blood serum should be used in making complement-fixation tests. However, if proper precautions are taken in the collection of specimens, there is no danger of bacterial contamination, and it is our experience that, unless such contamination occurs, a positive result will not be obtained in normal serum, even when kept for a considerable period of time. Personal experiments have demonstrated that blood sera may be kept at room temperature for as long as one month without danger of a negative serum becoming positive, provided there is no bacterial contamination in the serum. While, in such specimens, anticomplementary bodies frequently develop, after they have been kept for a week or more, these bodies cause inhibition of hemolysis in both the antigen and control tubes of the serum, and thus while destroying the value of the test, this type of reaction does not cause any confusion in the diagnosis of the disease. However, these observations emphasize the importance of collecting specimens for the Wassermann test under aseptic precautions if the sera can not be examined within 24 hours after collection.

The Influence of Variations in the Strength of the Reaction in Untreated Cases of Syphilis upon the Results of the Test.—During the routine examinations of blood sera for the Wassermann test, the writer has frequently observed cases in which the reaction varied from a strongly suspicious one to a plus-minus or negative one within periods of one or two weeks, while, in rare instances, a positive reaction would be found negative upon a second examination within the same period of time, no treatment having been administered at any time. In all such instances subsequent tests resulted in a positive reaction, so that it was evident that the variations in results, provided the technic of the test was the same, must have depended upon the reduction or absence of the body or bodies in the patient's serum which produce the reaction.

The fact that the blood serum of an undoubted syphilitic patient may, during certain intervals of time, give a negative reaction in the absence of specific treatment, and when previous and subsequent tests are positive, is of practical importance. While in the writer's experience such anomalous results have not been very numerous, their occurrence, together with the criticisms of the test by some authors who have had a similar experience, rendered an experimental investigation of the phenomenon of value, and in 1914, the writer[40] published certain observations upon the subject which will be briefly discussed at this time. The observations proved that marked daily variations occur in the strength of the complement-fixation reaction in the blood of syphilitics. While, *a priori,* there would appear to be no reason why the strength of the reaction should not vary, we have grown to consider the Wassermann reaction, if once obtained, as a stable quantity, the discordant results sometimes observed being imputed to the serologist or considered as proof of the unreliability of the test.

Ten patients suffering from undoubted syphilis were selected for the observations. Most of these men were

prisoners at the U. S. Military Prison, at Fort Leavenworth, and thus all chance of the reaction being influenced by the use of alcohol was obviated, the men being under a rigid discipline and on a plain, wholesome diet. Of the ten patients, two were in the primary stage of the disease, four in the secondary stage, and four in the latent stage. No treatment had been administered for over a year in any case, and then only to those who were in the latent stage. The patients in the primary stage had typical chancres and have since developed secondary eruptions; the secondary cases all presented eruptions and mucous patches; while the four in the latent stage were free from obvious symptoms. All gave double-plus (four-plus) Wassermann reactions at the time of the experiment.

The blood was collected every day and tested upon the same day it was collected, daily titrations of the strength of the complement fixation being made. The technic used was that employed by the writer for several years and which is described in this work.

Each patient's blood serum was titrated every day for one week, the quantities of serum employed being 0.02 c.c.; 0.04 c.c.; 0.06 c.c.; 0.08 c.c.; and 0.1 c.c. The quantity of blood serum used with the technic for diagnostic purposes is 0.1 c.c. so that this quantity may be taken as the diagnostic amount in the tables that follow. A control of 0.1 c.c. of the serum titrated was always employed.

In the tables it will be understood that the sign, ++, indicates absolute inhibition of hemolysis, or a positive reaction; the sign, +, anything between absolute inhibition and 50 per cent of inhibition of hemolysis; the sign, +−, anything between 50 per cent of inhibition and total hemolysis, or practically a negative reaction; and the sign, −, total hemolysis or a negative result.

Results in the Primary Stage of Syphilis.—Owing to lack of material, only two cases were tested in the primary stage of the disease. Both presented typical chancres and both gave a double-plus, or positive, Wassermann reaction

when first tested. Tables V and VI give the results of the daily titrations of the blood serum in these two cases.

TABLE V

RESULTS OF TITRATION OF BLOOD SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 1—PRIMARY SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM IN C.C.'S					CONTROL. 0.1 C.C.
	0.02	0.04	0.06	0.08	0.1	
Nov. 19	+–	+	+	+	+	–
Nov. 20	+–	+–	+	++	++	–
Nov. 21	+–	+	++	++	++	–
Nov. 22	+–	+–	+	+	+	–
Nov. 23	+	++	++	++	++	–
Nov. 24	+–	+	+	+	+	–
Nov. 25	++	++	++	++	++	–

TABLE VI

RESULTS OF TITRATION OF BLOOD SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 2—PRIMARY SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM. C.C.					CONTROL. 0.1 C.C.
	0.02	0.04	0.06	0.08	0.1	
Nov. 19	+	++	++	++	++	–
Nov. 20	+–	++	++	++	++	–
Nov. 21	–	+–	+	+	+	–
Nov. 22	–	+–	+–	+	+	–
Nov. 23	+	++	++	++	++	–
Nov. 24	+	++	++	++	++	–
Nov. 25	+	++	++	++	++	–

A study of these tables will show that considerable variations occurred in the complement-fixing power of the blood serum in both these cases of primary syphilis, especially in the smaller amounts employed, but that in neither case did the reaction become negative with the diagnostic amount of serum; i. e., 0.1 c.c. However, in one case a plus or doubtful reaction was obtained upon three of seven days, while in the other the same result was obtained upon two of the seven days. These results are very significant when it is remembered that in this stage of syphilis the Wassermann test is so often doubtful, or negative, and indicates that frequent examinations would give a much higher percentage of positive results than are usually re-

corded. Both of these cases, if tested upon certain days of the week, would have been reported serologically doubtful, but, fortunately, in most primary infections *Treponema pallidum* may be demonstrated with the dark-field, thus avoiding the confusion that might be caused by variations in the strength of the Wassermann reaction.

Results in the Secondary Stage of Syphilis.—The blood serum of four typical cases of secondary syphilis,

Table VII

Results of Titration of Serum for Complement-fixing Strength in Case 3—Secondary Syphilis

Date of test	Amount of blood serum. c.c.					Control c.c.
	0.02	0.04	0.06	0.08	0.1	0.1
Nov. 26	++	++	++	++	++	–
Nov. 27	++	++	++	++	++	–
Nov. 28	++	++	++	++	++	–
Nov. 29	++	++	++	++	++	–
Nov. 30	++	++	++	++	++	–
Dec. 1	++	++	++	++	++	–
Dec. 2	++	++	++	++	++	–

Table VIII

Results of Titration of Serum for Complement-fixing Strength in Case 4—Secondary Syphilis

Date of test	Amount of blood serum. c.c.					Control c.c.
	0.02	0.04	0.06	0.08	0.1	0.1
Jan. 3	++	++	++	++	++	–
Jan. 4	++	++	++	++	++	–
Jan. 5	++	++	++	++	++	–
Jan. 6	++	++	++	++	++	–
Jan. 7	+	++	++	++	++	–
Jan. 8	+	++	++	++	++	–
Jan. 9	++	++	++	++	++	–

Table IX

Results of Titration of Serum for Complement-fixing Strength in Case 5—Secondary Syphilis

Date of test	Amount of blood serum. c.c.					Control c.c.
	0.02	0.04	0.06	0.08	0.1	0.1
Dec. 24	+–	+–	+	++	++	–
Dec. 25	–	–	–	–	+–	–
Dec. 26	–	+–	+–	+	+	–
Dec. 27	–	+–	+–	+–	+	–
Dec. 28	+–	+	+	++	++	–
Dec. 29	–	+–	+–	+	++	–
Dec. 30	–	+–	+	+	++	–

TABLE X

RESULTS OF TITRATION OF SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 6—SECONDARY SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM. C.C.					CONTROL C.C.
	0.02	0.04	0.06	0.08	0.1	0.1
Dec. 5	+-	+-	+	+	++	-
Dec. 6	++	++	++	++	++	-
Dec. 7	++	++	++	++	++	-
Dec. 8	++	++	++	++	++	-
Dec. 9	++	++	++	++	++	-
Dec. 10	+-	+	+	+	+	-
Dec. 11	+-	+-	+	+	+	-

all showing lesions of the disease, was titrated in the same manner as described and Tables VII, VIII, IX, and X give the results obtained.

In Case 3 the strength of the blood serum did not vary at all during the time it was titrated, a double-plus (four-plus) reaction being obtained, even with the smallest amount of serum used. The case is of interest as showing how strong the blood serum is in complement-fixing power in some instances and how little it varies in strength even when very small amounts of serum are used. The patient presented clinically a very marked secondary eruption over the chest, abdomen, and back.

In Case 4, likewise, there was practically no variation in the strength of the reaction. In this case the patient presented mucous patches in the mouth and a well marked secondary eruption.

In Case 5, the variations in the strength of the complement-fixing power of the blood serum varied markedly, especially when amounts of serum of less than 0.1 c.c. were used. With the diagnostic amount of serum; i. e., 0.1 c.c., the serum gave a plus-minus, or practically negative result upon one day and a plus, or doubtful reaction upon two of the seven days during which it was tested. With 0.08 c.c. of the serum, an amount which was formerly used in the writer's laboratory as the diagnostic amount, a negative reaction was obtained upon one day and plus-minus upon one day and a plus, or doubtful reaction, upon two days.

In this case the Wassermann reaction was practically negative on one day and doubtful upon two days, although very marked secondary symptoms were present.

In Case 6 the variation in the strength of the reaction was not very marked, when the diagnostic amount of blood serum was used, the serum giving absolute inhibition of hemolysis upon all but two days, when it gave a plus or doubtful reaction.

The results of the titration of the blood serum in secondary cases demonstrate that variations do occur daily in cases showing well marked symptoms of the disease and that a case giving absolute inhibition of hemolysis upon one day may be negative or doubtful upon a succeeding day. When small amounts of blood serum are used the variations in the strength of the reaction are very marked, in some instances, as in Case 5, while in others the strength of the reaction remains unchanged. (Case 3.)

Results in the Latent Stage of Syphilis.—Four patients in the latent stage of syphilis were tested, all of whom gave a history of definite symptoms of the disease, but in none had symptoms been present within one year. All of the men had received one or two injections of salvarsan and all gave a double-plus (four-plus) Wassermann reaction shortly before the titrations of the blood serum were commenced. Tables XI, XII, XIII, and XIV give the results of the titrations.

Table XI

Results of Titration of Serum for Complement-fixing Strength in Case 7—Latent Syphilis

Date of Test	Amount of Blood Serum. c.c.					Control c.c.
	0.02	0.04	0.06	0.08	0.1	0.1
Dec. 19	+–	+	+	++	++	–
Dec. 20	–	+–	+	+	+	–
Dec. 21	–	+	++	++	++	–
Dec. 22	–	+–	+	+	+	–
Dec. 23	++	++	++	++	++	–
Dec. 24	++	++	++	++	++	–
Dec. 25	+–	+	+	+	+	–

TABLE XII

RESULTS OF TITRATION OF SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 8—LATENT SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM. C.C.					CONTROL C.C.
	0.02	0.04	0.06	0.08	0.1	0.1
Jan. 12	+	++	++	++	++	–
Jan. 13	+	++	++	++	++	–
Jan. 14	+	++	++	++	++	–
Jan. 15	+–	+	++	++	++	–
Jan. 16	+–	+	++	++	++	–
Jan. 17	+–	+	++	++	++	–
Jan. 18	+–	+	+	+	++	–

TABLE XIII

RESULTS OF TITRATION OF SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 9—LATENT SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM. C.C.					CONTROL C.C.
	0.02	0.04	0.06	0.08	0.1	0.1
Jan. 15	–	+–	++	++	++	–
Jan. 16	–	+–	+	+	+	–
Jan. 17	+–	+	+	++	++	–
Jan. 18	–	–	+–	+	++	–
Jan. 19	–	–	–	–	–	–
Jan. 20	–	–	+–	+	++	–
Jan. 21	–	–	–	+–	+–	–

TABLE XIV

RESULTS OF TITRATION OF SERUM FOR COMPLEMENT-FIXING STRENGTH IN CASE 10—LATENT SYPHILIS

DATE OF TEST	AMOUNT OF BLOOD SERUM. C.C.					CONTROL C.C.
	0.02	0.04	0.06	0.08	0.1	0.1
Jan. 15	–	–	–	+–	+	–
Jan. 16	–	+–	+	+	++	–
Jan. 17	–	+–	+	+	+	–
Jan. 18	–	–	+–	+	+	–
Jan. 19	–	+–	+–	+–	+–	–
Jan. 20	–	+–	+–	+	++	–
Jan. 21	+–	++	++	++	++	–

In Case 7 the strength of the complement-fixation reaction, using the diagnostic amount of serum, i. e., 0.1 c.c., was only sufficient upon three of the seven days that the serum was tested to give a plus or doubtful reaction, while with smaller amounts the variations in the strength were very marked. Upon these three days the test would have been reported as doubtful and if further tests had not been

made, the diagnosis, owing to the absence of symptoms, would have been missed.

In Case 8 there was no variation from a positive reaction with the diagnostic amount of serum during the seven days the titrations were made, although some variation occurred with smaller amounts of the serum.

In Case 9 there were very marked variations in the strength of the reaction with the diagnostic amount of serum, a negative reaction being obtained upon one day, a plus-minus upon another, and a plus or doubtful reaction upon a third. A double-plus, or positive reaction, was obtained upon only four of the seven days that the serum was tested. With amounts of serum smaller than the diagnostic amount of 0.1 c.c. the variations were very great.

In Case 10 there was also great variation, from day to day, in the strength of the complement-fixation reaction, the diagnostic amount of serum giving a plus-minus reaction upon one day, a plus reaction upon three days, and a double-plus or positive reaction upon three days of the week. With smaller amounts of serum the variations were still more marked.

General Discussion.—The most important practical point brought out by these observations on the daily titration of the blood serum of untreated syphilitics is that great variations may occur in the complement-binding power of the serum of patients uninfluenced by any kind of treatment, and that these variations occur from day to day, so that one negative examination, or even more, in a suspected case, is of absolutely no value in excluding the disease. In several of the cases mentioned the blood serum, if tested upon certain days only, would have given a negative, or practically negative, result, although serum from the same case had been previously positive, and again became positive within a day or two. If this be true of untreated cases, some of them showing severe lesions of the disease, it will certainly be found true of a greater number of latent and treated infections, the class of cases in which a negative Wassermann

reaction is so often considered decisive as to the absence of the disease. The results of these tests indicate the great value of repeated examinations, when a negative Wassermann is reported, before a patient is considered as cured, or before he is assured that he is not suffering from a syphilitic infection.

As to the cause of the variations in the strength of the reaction observed from day to day, it is evident that the antibodies and lipotropic substances present in the patient's serum must vary from day to day, or such results would not be obtained. It may be accepted as proved that there exist in the blood of syphilitics antibodies and lipotropic substances that are capable of fixing complement in the presence of a proper antigenic extract, and that these bodies depend for their origin on the presence in the body of living treponemas, for when the latter disappear from the lesions in experimental animals, as the result of treatment, the complement-fixation reaction also disappears. Whether these substances are produced by the reaction of the tissues to endotoxins liberated by the breaking down of dead treponemas; to toxins produced by the living treponemas; or to the presence in the blood of the toxins themselves; are all mooted questions, but these experiments prove that there must be a certain amount of them in the blood serum before a positive Wassermann reaction can be obtained, and that this amount varies considerably from day to day.

It is but just to call attention to the importance of these results in explaining, possibly, the discrepancies between Wassermann reports from various laboratories where specimens of blood from the same individual were examined at different times, for it is evident that unless the same specimen of blood be examined no reliable conclusions can be drawn regarding the reports of different laboratories, a fact that should be borne in mind when it is desired to obtain a report from more than one laboratory upon a suspected individual.

The factors which have been mentioned in this chapter as markedly influencing the results of the Wassermann test; i. e., the effect of alcohol; of bacteria growing in the blood serum to be tested; and normal variations in the power of the blood serum to bind complement, are all of practical importance and should all be considered in performing the test and in judging of its results in any case where there is a question of the accuracy of the reaction. If this were done, there would be much less criticism of the Wassermann test than there is, and a much better understanding of its reliability and its limitations.

CHAPTER IV

PREPARATION AND TITRATION OF THE REAGENTS USED IN THE WASSERMANN TEST

The following list of apparatus and reagents needed in performing the Wassermann test is here inserted and it will be found to include all that is really essential for this work:

List of Apparatus

1 Luer syringe, capacity 1 c.c.
1 Luer syringe, capacity 5 c.c.
1 Luer syringe, capacity 20 c.c.
6 needles, for collecting blood, (special needles for this purpose can be obtained of any good surgical supply house).
1 electric centrifuge, International Instrument Co., Size 1, Type A, with one four 15 c.c. tube head and metal holders, and one four 50 c.c. tube head and metal holders. Type of current and voltage must be specified.
20 centrifuge tubes, plain, 15 c.c.
10 centrifuge tubes, graduated, 15 c.c.
10 centrifuge tubes, 50 c.c., plain.
1 incubator, either electric, gas, or oil, with proper thermoregulators, to run at 56° C.
1 water-bath, with thermometer, burner and thermoregulator, large enough to accommodate the required number of Wassermann racks, to run at 37° C.
1 set of 20 copper test tube racks, made by Topham, Washington, D. C.
1 amboceptor cutter, made by Topham, Washington, D. C.
1 set of 3 amboceptor markers, 4 mm., 5 mm., and 6 mm., made by Topham, Washington, D. C.
1 paraffin bath to fit incubator mentioned above, to hold tubes during inactivation of blood serum.
100 or more test tubes, without lip, 100 by 12.5 millimeters.
4 test tube baskets, 6 inches high by 7.5 inches in diameter.
10 Mohr pipettes, 10 c.c. capacity, graduated in tenths.
10 Mohr pipettes, 5 c.c. capacity, graduated in tenths.

20 pipettes, serologic, 1 c.c. capacity, graduated in hundredths to tip. These pipettes should be of small bore and the graduations far enough apart to render accurate readings possible for quantities as small as one-one-hundredth of a c.c.

100 or more pipettes, serologic, capacity 1 c.c., graduated in tenths. These are used for measuring the sera to be tested.

12 Erlenmeyer flasks, capacity 250 c.c. each.

6 animal cages.

20 pounds soft paraffin, melting point 43° C.

1 bottle, 500 gm. sodium chloride, C. P.

1 bottle, 30 gm. cholesterin, Merck's.

1 bottle, 500 gm. absolute alcohol.

6 triangular files, 3 inches long.

Guinea pigs and rabbits must be kept for complement and amboceptor production and a small and suitable room or animal house should be set aside to shelter them and to allow for breeding.

Preparation of Reagents

The proper preparation of the reagents used in the Wassermann test is of great importance and explicit directions are given in the following pages regarding this subject, which, if carefully adhered to, will result in the preparation of reagents that will give accurate results.

The Wassermann test as performed in the writer's laboratory and in most of the laboratories connected with the Medical Department of the Army, is a modification of both the test as originally recommended by Wassermann, and the modification of that test recommended by Noguchi. The technic was originally worked out by the writer and has since been standardized, so far as the apparatus is concerned, by Lieut. Colonel Vedder, of the Medical Corps. A human hemolytic system is employed instead of the sheep system recommended by Wassermann; alcoholic extracts of both foetal syphilitic liver, and cholesterinized alcoholic extracts of normal human heart muscle, are used as antigen; and the blood serum is inactivated at 56° C. for

one-half hour before it is tested. The following reagents are employed in the test:

1. *Complement.* The fresh blood serum of guinea pigs.

2. *Hemolytic amboceptor.* The blood serum of rabbits immunized to human red blood corpuscles.

3. *Hemolytic antigen.* A suspension of human red blood corpuscles.

4. *Syphilitic antigen.* An alcoholic extract of foetal syphilitic liver and a cholesterinized alcoholic extract of normal human heart muscle.

5. *Syphilitic amboceptor.* The patient's blood serum. This may or may not contain the lipotropic substance or substances necessary for complement fixation with the antigens mentioned.

Preparation of Complement.—Complement is a constituent of the fresh blood serum of all animals but certain animals furnish better complement for complement-fixation tests and for the Wassermann test than do others, and it has been demonstrated that for the Wassermann test the blood serum of the guinea pig supplies a better complement than does that of other animals. Noguchi and Bronfenbrenner tested the blood serum of the sheep, dog, ox, guinea pig, hog, and rabbit, and found that the serum of the guinea pig gave the best results when used as complement in the Wassermann test, so that the blood serum of this animal has been generally adopted for this purpose.

The blood serum of different guinea pigs varies considerably in complementary activity, even in health, and in unhealthy animals, and when the temperature is either very hot or cold, marked variations may occur. For this reason it is essential that the blood serum be titrated for its complementary power each time that it is used, and it is always best, in practice, to employ the serum from two or three guinea pigs, mixed, rather than to depend upon that of a single pig, which might be found to be so weak as to be inconvenient for use in the test.

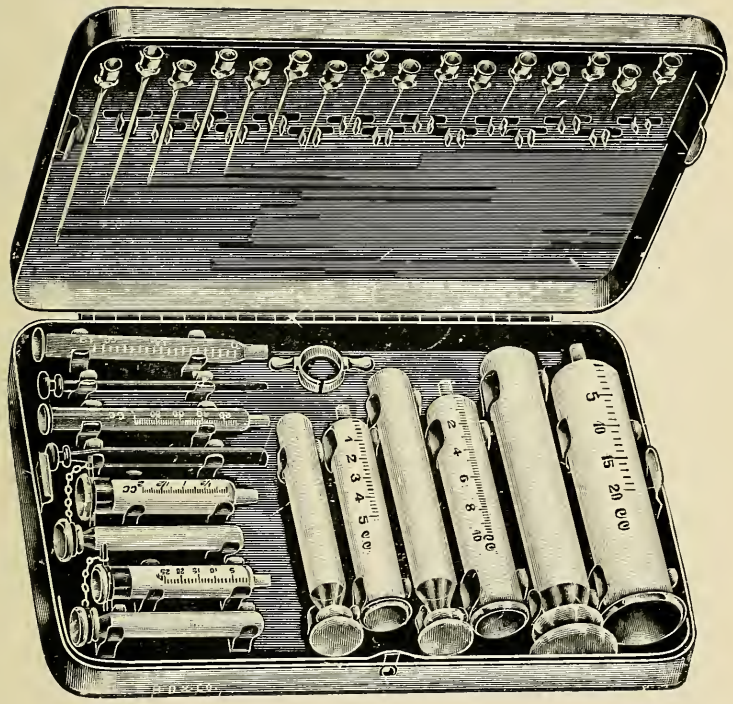

Fig. 3.

Luer syringes. Set of seven, suitable for use in the Wassermann test, and for serologic work in general.

The guinea pig may be either bled from the heart or its throat cut. It is the practice, in the writer's laboratory, to bleed the pigs from the heart, as by this method the animal is not sacrificed and can be used over and over again for bleeding, provided the bleedings be from three to four weeks apart. Several lots of pigs can be rotated and thus considerable expense saved, as the animals usually stand the bleeding well and it is seldom that any are lost if the bleeding is properly done. The operation should be performed under ether and consists simply in entering the heart with a hypodermic needle attached to a syringe holding 10 c.c. and nearly this amount of blood can be aspirated from the heart without danger to the animal. It is well to connect the needle of the syringe to the syringe with a flexible rubber tube, as it is much easier to make the puncture and handle the syringe afterward if this is done. The operation requires considerable practice but when the technic is once mastered it is by far the most satisfactory way of obtaining complement for the Wassermann test, especially if only a small number of tests are to be made at one time.

After bleeding, the blood should be ejected from the syringe into a sterile Petrie dish, allowed to stand at room temperature for two hours and then placed in the ice box overnight. In the morning the clear serum is pipetted off and if it is to be used at once, diluted with an equal amount of normal salt solution—(0.85%). If it is not to be used for several hours it should be placed at once in the ice box, as the complementary power of blood serum is weakened by exposure to room temperature for any great length of time. The serum should be free from blood corpuscles and almost colorless or but very slightly blood stained.

If a very large number of tests are to be made it may be better to sacrifice the pigs by cutting their throats, each pig being held over a Petrie dish, the throat cut quickly with a sharp scalpel or razor, after shaving the neck, and the blood allowed to run into the dish. Great care should

be taken not to cut too deeply, so as to penetrate the esophagus or trachea, as the secretions from the stomach have a marked inhibitory effect upon complement and this is also true to a lesser degree of the secretions of the larynx or trachea. After collecting, the blood should be treated in the same manner as after puncture of the heart.

The syringe, needles, Petrie dishes, and all apparatus used in collecting the blood for complement should be sterile and perfectly dry, with the exception of the syringe, which should be washed out with normal salt solution before the puncture is made. If the blood serum, after being pipetted off, is much blood stained, it should not be used for complement. If it contains blood corpuscles these may be removed by centrifuging and pipetting off the clear serum above the layer of corpuscles at the bottom of the centrifuge tube.

The complement, even after being diluted one-half with normal salt solution, may be kept in the ice box without much loss of strength for about three days, but usually upon the third day the titration of it will show such loss of complementary power that it will be useless for the test. Complement appears to be at the height of its complementary power about thirty-six hours after removal from the pig, but is much weaker immediately after withdrawal of the blood and grows gradually weaker from day to day after this period.

Preservation of Complement.—Many attempts have been made to preserve complement by the addition of various substances, but none of the methods so far advocated have been generally adopted. Noguchi[41] attempted to preserve it by impregnating filter paper with it, a method very successful with amboceptor serum, but without satisfactory results. Kolmer[42] recommends chemically pure sodium chloride as the best preservative, adding 0.425 gram sodium chloride to each 10 c.c. of sera obtained from several guinea pigs. He states that complement preserved in this

manner will maintain its hemolytic and fixing properties for several weeks.

Preparation of Hemolytic Amboceptor.—The hemolytic serum or amboceptor used in the Wassermann test as performed by the writer is prepared by immunizing rabbits to human red blood corpuscles. Noguchi[43] was the first to call to the attention of serologists the advantage of a human hemolytic system in Wassermann work and the writer believes that it is the system best suited for complement-fixation reactions upon the blood of man. Its adoption obviates the danger that is present in human blood serum, of the action of the natural antisheep amboceptor normally present in such blood serum, and which, when the sheep hemolytic system is employed, should be removed from such serum before the test is made. It has not been practicable, owing to the immense number of Wassermann tests made in army laboratories, to use any method which removes this natural antisheep amboceptor from blood serum, and, therefore, aside from the superiority of the human hemolytic system for complement-fixation work, it was necessary to adopt it from practical considerations. The only objection that can be urged against the human system is that it is more difficult to immunize rabbits against human erythrocytes than against sheep corpuscles and that it requires a more careful technic both in collecting the blood and injecting it into the animals, but this objection is of no weight when the great advantages of the human hemolytic system are considered.

Before considering the method of preparing the hemolytic amboceptor serum there are certain general principles regarding amboceptors, either bacteriolytic or hemolytic, that will be mentioned.

It will be remembered that amboceptors are produced in the blood serum of an animal in response to the injection of an antigen, and that these amboceptors are specific for the antigen injected. If the blood serum of the animal containing them is added to a mixture of the antigen and of

fresh blood serum, or complement, the amboceptor will act as an intermediary body, allowing the complement to act upon the antigen, and bacteriolysis or hemolysis will result.

Amboceptors are thermostable; i. e., their activity is not destroyed by heating the serum containing them for one half hour at 56° C. They are also resistant to a considerable degree to acids and alkalies, and to drying. A good hemolytic serum can be preserved upon filter paper, in a dry condition, for many months with very little loss of strength and this fact is made use of in the Wassermann test, as will be noted later.

Amboceptors appear to act as a chemical linking body, connecting the antigen with the complement, as the complement is unable to act upon an antigen except through the agency of the amboceptor. Hemolytic amboceptors occur in very small amounts in the blood of normal animals and such amboceptors are sometimes present in an amount sufficient to cause trouble in complement-fixation reactions, as in the case of the normal antisheep amboceptor present in human blood serum.

In producing hemolytic amboceptor for human red blood corpuscles in rabbits several methods have been recommended which will be described. The writer has obtained the best results with the method first described but the other methods are given in order that a choice may be made if desired, according to circumstances.

Well grown, perfectly healthy rabbits are selected, preferably pure white ones, and are given repeated injections of washed human red blood corpuscles. The blood corpuscles are prepared for the injections in the following manner: The requisite amount of blood, allowing at least twice the amount of blood as the amount of corpuscles to be injected, is withdrawn from one of the large veins of the arm with a glass syringe, which has been sterilized and washed out with sodium citrate solution. After drawing the blood, it is at once ejected into a flask containing from 200 to 250 c.c. of normal salt solution (0.85%), and dis-

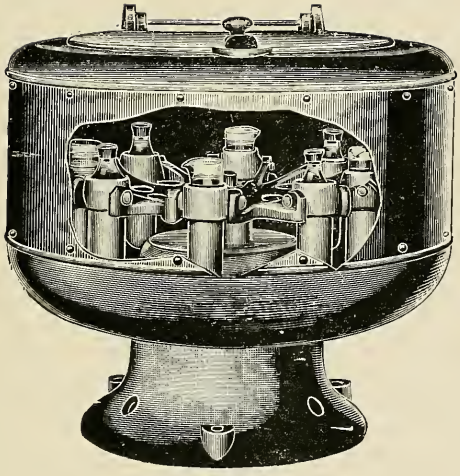

Fig. 4.
Electric centrifuge.

tributed into large centrifuge tubes (50 c.c.) and centrifuged until the erythrocytes are deposited and the supernatant fluid is practically colorless. The supernatant fluid is now poured or pipetted off and the tubes again filled with the salt solution, well shaken so as to distribute the red cells throughout the solution, and again centrifuged. This process is repeated four times, when the supernatant salt solution is tested for albumin; if the merest trace is demonstrated, the washing is again repeated and continued until no reaction for albumin is obtained. Almost invariably four washings will be found sufficient. Care should be taken not to centrifuge for so long that the corpuscles become packed at the bottom of the tubes, for the force necessary to dislodge them will break up a certain percentage and render them unsuitable for injection. Care should also be taken to have each centrifuge tube weigh the same as all others, and this can be done by weighing each in a balance at the time they are filled. Unless this is done the centrifuge will run unevenly and is liable to breakage or accident.

After the corpuscles are washed in the manner described they are mixed with as little normal salt solution (0.85) which must be sterile, as is necessary to secure a suspension that will pass through the needle of the injection syringe, and are at once injected, either intravenously or subcutaneously, with a sterilized syringe.

Methods of Securing a Good Amboceptor Serum.—The methods of securing a good amboceptor serum vary somewhat but the writer has found that the most rapid method of immunizing the rabbits and securing a good hemolytic serum is to give an intravenous injection, in the ear vein, of 1 c.c. of washed erythrocytes every other day until five or six injections have been given, when the rabbit's blood serum should be titrated and will generally be found strong enough in hemolytic properties to be used for the test. If it is not, two or three more intravenous injections of the same amount of red corpuscles should be given. Rarely,

animals are found that will not develop a sufficiently strong serum, no matter how many injections may be given, but usually from five to six injections will be found sufficient. By this method it is very seldom that an animal is lost, as the frequent injections prevent the development of anaphylaxis, and a good serum for use as amboceptor in the Wassermann test can be secured within a comparatively short period of time.

Another good method is to give one subcutaneous injection of 5 c.c. of washed human erythrocytes in the abdomen, followed by from three to four intravenous injections in the marginal vein of the ear of from 3 to 4 c.c. of washed erythrocytes, at intervals of from five to six days. This method is much slower than the preceding, and not infrequently an animal is lost through anaphylactic phenomena.

Noguchi[44] recommends the following method of producing hemolytic amboceptor for the Wassermann test: Five injections of washed human erythrocytes are given subcutaneously in the abdomen, the first of 5 c.c.; the second, 8 c.c.; the third, 12 c.c.; the fourth, 15 c.c.; and the fifth, 20 c.c.; at five day intervals. Nine days after the last injection the rabbit is bled for a small amount of blood from the ear vein and the serum titrated. This method is an excellent one but consumes much more time than the method of intravenous injections made every other day, recommended above.

Whatever method is used for immunizing the rabbits, the blood serum should not be tested for its hemolytic properties until from 7 to 9 days have elapsed from the date of the last injection of red corpuscles.

Preservation of the Amboceptor Serum.—The amboceptor serum may be preserved in ampules holding from 1 to 5 c.c. in the ice box, and used as required, but the writer has employed for nearly ten years the method of preserving amboceptor serum for the Wassermann test recommended by Noguchi with the most satisfactory results and

this method is used in most of the army laboratories. It consists in impregnating suitable filter paper with the blood serum, drying, and storing in a glass container in a dark closet or drawer. The method has entirely replaced the use of liquid amboceptor in our laboratories and preserved in this manner the serum keeps much better, and preserves its strength for a much longer time, than when it is kept in liquid form, and the danger of bacterial contamination, always present with the liquid serum, is avoided.

Before impregnating the filter paper, a preliminary titration of the rabbit's blood serum should be made in order to determine whether it is strong enough in hemolytic amboceptor to use in the test. For this purpose a small amount of the rabbit's blood is collected from the ear vein in a capillary pipette or Wright tube and the serum allowed to separate. It is then separated from the clot and inactivated by heating it for one-half hour at 56° C. in the paraffin bath or water-bath, and one drop of it, from a capillary pipette, is mixed with 39 drops of sterile normal salt solution (0.85) and titrated for hemolytic strength. The method of titration is illustrated in Table XV.

TABLE XV

PRELIMINARY TITRATION OF AMBOCEPTOR SERUM FOR WASSERMANN TEST*

TUBE NO.	AMOUNT OF SALT SOLUTION. 0.85%. C.C.	NUMBER OF COMPLEMENT UNITS	AMOUNT OF BLOOD SUSPENSION. 5%. C.C.	AMOUNT OF AMBOCEPTOR SERUM, DILUTED 1-40. DROPS	
1	0.9	1	0.1	1	Incubate in water-bath at 37° C. for one hour or in incubator at same temperature for two hours and read results.
2	0.9	1	0.1	2	
3	0.9	1	0.1	3	
4	0.9	1	0.1	4	
5	0.9	1	0.1	5	
6	0.9	1	0.1	None	

*If the unit of complement has not been determined by a previous titration, use 0.05 c.c. of a 1:2 dilution in salt solution.

After incubating in the water-bath at 37° C. for one hour, or in the incubator for two hours, the titration is

read, and if either of the tubes containing one or two drops of the rabbit's serum shows complete hemolysis, the serum is strong enough in hemolytic amboceptor for use in the test and the rabbit should be bled at once. The control tube, No. 6, should show complete inhibition of hemolysis.

Bleeding is accomplished most quickly by cutting the carotid vessels while holding the rabbit over a large glass dish, into which the blood is allowed to flow. The neck of the animal, where the incision is to be made, should be shaved and washed carefully but no disinfectant should be used. The glass dish to contain the blood should be sterilized before use and after the blood has been collected it should be kept at room temperature for about two hours and then placed in the ice box overnight. In the morning the clear serum should be pipetted off into small test tubes, inactivated by heating it for one-half hour at 56° C. in the paraffin or water-bath, and then kept in the ice box until one is ready to impregnate the filter paper. It does no harm if the blood serum is slightly tinged with hemoglobin, as the amount used in the test is so small that it will not interfere with the reading of the reaction.

Impregnating the Filter Paper.—The filter paper used for this purpose is Schleich and Schull's No. 597, but any other make of paper of approximately the same texture will serve equally as well. The paper is cut into squares measuring 10 by 10 centimeters, and actual experience has shown that it will take about 1.5 c.c. of the rabbit serum to saturate one of these squares of paper, so that it is an easy matter to calculate how many squares will be required for any given amount of the serum. This number is placed, one by one, in a suitable Petrie dish or other glass dish containing the serum, and the process continued until all the serum is absorbed by the paper. The squares of saturated paper are then carefully lifted with a pair of forceps, thoroughly drained, and drawn across the edge of the dish a few times to remove any excess of serum, and then placed upon a piece of unbleached muslin, which

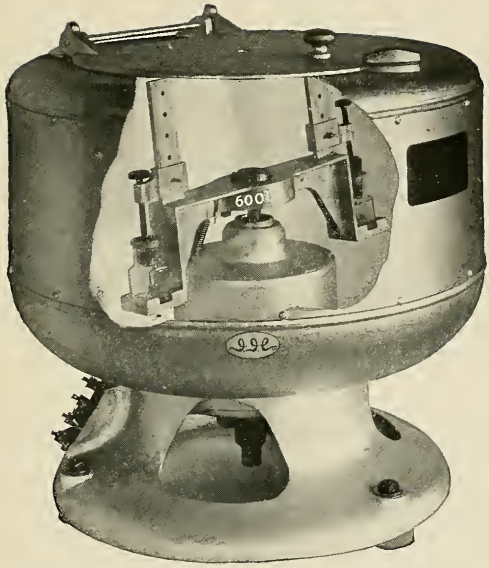

Fig. 5.
Same centrituge illustrated in Fig. 4, with shaker head attached, for use in making antigenic extracts.

should be large enough to contain all the slips of paper. The muslin containing the paper is now placed under an electric fan and the paper rapidly dried, after which it should be placed in air-tight glass containers and kept at room temperature in a dark and dry place. Before use, it should be titrated as will be described.

The amboceptor paper thus prepared will retain its working strength for many months, but it should be titrated at least once a month in order to avoid any sudden loss of strength, although the writer has never observed such losses in the many years that he has used the amboceptor paper. Paper over a year old has been repeatedly used and in practically the same dose as when it was first prepared and paper two years old was found to have lost only one-eighth of its original strength at the end of that time.

Preparation of the Hemolytic Antigen.—The hemolytic antigen used in the test consists of a 5 per cent suspension of human red blood corpuscles, prepared in the following manner:

In the Wassermann test, as recommended by the writer, each tube contains 0.1 c.c. of a 5 per cent suspension of human red blood corpuscles in 0.85 per cent salt solution. The quantity of blood required for a series of tests will depend, of course, upon their number. One cubic centimeter of blood will furnish enough suspension for nearly one hundred tests, and unless one is doing a very large number of tests, enough blood may be easily secured by constricting the middle finger, after forcibly swinging the arm about for a few times, with a rubber tube, and pricking the finger just above the root of the nail with a sterile needle or glass point. The blood is allowed to drop into sterile graduated centrifuge tubes, each tube being filled to the 9 c.c. mark with sterile normal salt solution (0.85%), and enough blood is added to fill the tube to the 10 c.c. mark. The tubes are then shaken well and centrifuged until all the blood corpuscles are collected at the bottom. The su-

pernatant liquid is then carefully poured, or pipetted off, the tubes again filled with salt solution, and again centrifuged until the cells are at the bottom of the tube. This process of washing is repeated until the corpuscles have been washed four times, which will be found sufficient.

The supernatant fluid is poured or pipetted off for the last time, and the amount of corpuscles at the bottom of the tube noted, and the tube filled with enough salt solution to make a 5 per cent suspension. Thus, if there are 0.5 c.c. of red corpuscles at the bottom of the tube, it would require 9.5 c.c. of salt solution to make a 5 per cent suspension. If only a small amount of blood is needed for a few tests, one centrifuge tube will be all that is required for the washing but if many tests are to be performed, the washing should be done with a greater number of tubes.

In the test, 0.1 c.c. of this suspension is added to each tube, which contains 0.9 c.c. of normal salt solution, thus making a half of one per cent suspension. This makes a suspension sufficiently strong to render the reading of the test easy and is much more saving of complement and amboceptor than the use of a stronger suspension.

Preparation of Antigens for Wassermann Test.—The *syphilis antigen,* as it may be called, as already stated, is not a true antigen, in the strict use of the term, as it is an extract containing lipoids which react with lipotropic substances in the blood serum of the syphilitic patient, but as it acts like a true antigen in the Wassermann test, it is convenient to retain the name, but it should be understood that it is not a real antigen, as are the red blood corpuscles used in the hemolytic system just described.

It is certainly true that in all alcoholic extracts of normal tissues used as antigens in the Wassermann test, the active principle is contained within lipoids in the extracts and that there is nothing specific as regards *Treponema pallidum,* in these extracts. When alcoholic extracts of foetal syphilitic liver, and especially watery extracts of such livers, are used as antigens, the extracts, besides con-

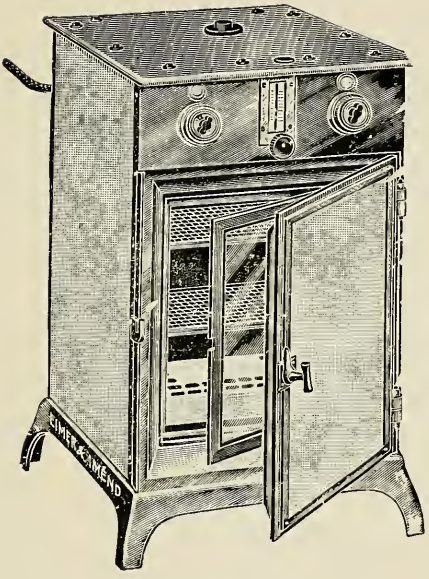

Fig. 6.
Small incubator, to run at 56° C., for inactivating sera.

taining the lipoids present in the normal extracts, may also contain specific antigenic substances due to *Treponema pallidum,* as has already been shown. However, even in such extracts, the greater part of the reaction is undoubtedly due to the lipoids present in them, although it can not be denied that in some instances, where the extracts of normal tissue give a negative or doubtful result, the extracts of foetal syphilitic liver and of cultures of *Treponema pallidum* give a positive one. Accordingly, the writer advises the use of both a cholesterinized alcoholic extract of normal human heart muscle and an alcoholic extract of foetal syphilitic liver in performing the test. If both can not be obtained the best results will be secured by using cholesterinized alcoholic extracts of normal tissues, preferably human heart muscle. The extracts of pure cultures of *Treponema pallidum,* while giving interesting results from a scientific standpoint, are useless as antigen in routine Wassermann work because of their unreliability. In practical work the writer recommends the antigens already mentioned, and, in addition, Noguchi's acetone-insoluble lipoid antigen, where the other antigens fail in apparently true syphilitic infections, but only in such infections.

Alcoholic Extract of Foetal Syphilitic Liver.—A foetal liver, rich in *Treponema pallidum* is selected, washed free from blood, and any fat present removed. One hundred grams is weighed out, all blood removed by washing, and cut into very small pieces. The material is then placed in a suitable bottle, or other container, and 1000 grams of absolute alcohol, or 95% alcohol, added, and the whole placed in an incubator at 37° C. and allowed to remain for 10 days, being thoroughly shaken three times a day during that time, when it will be ready to titrate. If a shaking machine is available, the material, after adding the alcohol, is placed in a suitable bottle or bottles, and shaken for 24 hours at a good rate of speed, at the end of which time it will be found that the tissue is thoroughly extracted.

After extraction by either method is completed, the material is filtered through filter paper and the filtrate titrated. If the first titration is not satisfactory, the filtrate should be evaporated to two-thirds its original volume and again titrated. The antigenic extract should always be kept in the ice box except when in use.

Cholesterinized Human Heart Extract.—The same method is used in preparing the alcoholic extract of heart muscle, the material being washed free from blood, all fat removed, and 100 grams of the heart muscle being cut into small pieces and extracted with alcohol in the same proportions (1000 grams of alcohol to 100 grams of muscle). After extraction one-half of the material is filtered through paper, and 0.4% of cholesterin added, and titrated. If the titration does not result satisfactorily as regards the antigenic strength of the extract, the remaining half is evaporated to two-thirds its original volume, again filtered through paper, and the cholesterin added, after which it is titrated. The amount of cholesterin added gives practically a saturated solution and there is generally an excess of cholesterin left after standing, and in using the antigen care should be taken not to get any of this excess into the pipette used in the work and thus in the diluted solution of the antigen. After the addition of the cholesterin the antigenic extract is placed in glass-stoppered bottles and kept in an ice box, except when in use.

Noguchi's Acetone Insoluble Lipoid Antigen.—This type of antigen was recommended by Noguchi when he evolved his modification of the Wassermann test and it is an excellent antigen, especially for use in cases in which the ordinary antigens described give doubtful results, as it appears to be more delicate than the extracts already described. However, it is true that sometimes this antigen appears to give positive reactions in cases that are negative with other antigens and in which syphilis can not be stated to exist upon clinical grounds, so that, in the writer's opinion, the use of this antigen should be restricted to

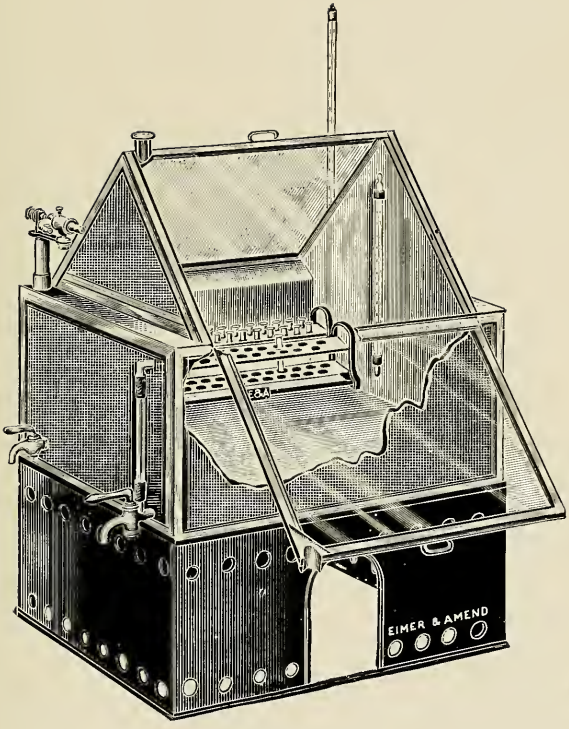

Fig. 7.
Water-bath, suitable for Wassermann test incubations.

the class of cases mentioned and as a control of other antigens, when doubtful results are obtained. It is prepared in the following manner:

Normal organs are used and may be from man or the lower animals. Extracts made from heart muscle, liver, or kidney of man or the same organs of the guinea pig or rabbit are satisfactory and the extraction is done in the same manner as for the antigens already described, 10 parts of absolute alcohol being used for each part of tissue. After extraction, the material is filtered, and the filtrate evaporated to dryness by the aid of an electric fan. The residue is taken up with ether and the solution thus obtained is allowed to stand over night in a cool place. In the morning the supernatant ether will be found perfectly clear while the insoluble portion of the residue has settled to the bottom of the receptacle. The clear etheral portion is decanted off and evaporated to a small quantity and mixed with ten volumes of pure acetone. The precipitate which forms is allowed to settle and the supernatant liquid poured off. The precipitate is light brown in color and forms the material from which the antigen suspension is made, 0.3 gram of this acetone insoluble fraction being dissolved in 1 c.c. of ether and then mixed with 9 c.c. of methyl alcohol. This mixture may be kept for a long time and the antigen suspension used in making the test is prepared by adding 1 c.c. of this stock solution to 9 c.c. of normal salt solution, or if less is needed at the time of making the tests, the same proportion of antigen and salt is preserved.

Wassermann's Aqueous Extract Antigen.—The antigen employed by Wassermann in his method of performing the complement-fixation test for syphilis was prepared as follows:

A foetal syphilitic liver, rich in treponemas, is cut into very small pieces and weighed. Four times its weight of 0.5 per cent phenol in physiologic salt solution is added and the mixture placed in a brown bottle and shaken in a shaking machine at room temperature for twenty-four

hours. It is then filtered through gauze and placed in a brown bottle in the ice box for several days, after which it is titrated to determine its antigenic strength. A sediment forms in the bottle after standing in the ice box and one should be careful not to disturb this in using the antigen.

This antigen is not satisfactory, as it varies very greatly in strength when made from different livers, and is very prone to lose strength rapidly, especially if it is left for any length of time at room temperature. It is not nearly as satisfactory as the antigens heretofore described.

Various other methods of preparing antigenic extracts for the Wassermann test have been described by Marie and Levaditi,[45] Landsteiner, Müller, and Pötzl,[46] Porges and Meier,[47] Browning, Cruickshank, and McKenzie,[48] and others, but none of them are better in practical use than those which have been treated of in the foregoing paragraphs.

Cholesterinized Antigens.—Before concluding this portion of our subject it may be well to discuss briefly the subject of the use of cholesterinized antigens in the Wassermann test. The writer advocates their use, or, at least, the use of an extract of human heart muscle, cholesterinized, because he has found by long experience that such an extract is perfectly reliable and does not give false positive reactions, as shown by the results in the many thousands of tests in which he has used such an extract. Some authorities claim that, in their experience, cholesterinized extracts are so very sensitive that they may give false positive results but this has not been the writer's experience. Very rarely there has been observed a plus or plus-minus reaction in apparently normal serum, but the writer has never observed a distinctly positive reaction with the cholesterinized heart extracts used in his method in a serum which could be proved to be normal, but, on the other hand, numerous instances have been observed in which ordinary extracts gave doubtful or negative results but in which the cholesterinized heart extract gave positive re-

actions. This is very apt to be the case in cases of syphilis that have been treated and, in the writer's opinion, the cholesterinized extract is far superior, as a control of treatment, than are extracts of normal tissues or of foetal syphilitic liver, to which cholesterin has not been added.

Titration of Reagents Used in the Wassermann Test.

The accurate titration of the various reagents used in the Wassermann test is of fundamental importance, as the test is essentially a quantitative one and the smallest variation in the amount of the reagents used may cause very great variations in the result of the test. If the amount of complement or amboceptor be excessive the strongest positive serum may give a negative result, while if the amount of these reagents is too small, normal sera will give positive results. The same is true of the amount of antigen, for an excess of antigen will result in a positive result in normal sera or in the blood sera from other diseases than syphilis, while too little will result in negative reactions in what should be found to be strongly positive sera.

It is easily seen, therefore, that the accurate titration of complement, amboceptor, and antigen is of the greatest practical importance, and the neglect of such titrations will be sure to lead to erroneous results and to great injustice being done the patient who has entrusted the testing of his blood to the laboratory.

Titration of the Complement.—The amount of complement to be used in the Wassermann test is of vital importance and is determined by a titration of the guinea pig serum each day before the tests are made. Some authorities employ exactly one unit of complement with one unit of amboceptor for each test but this allows of no inhibitory effect by either the blood serum or antigen, and, is therefore, a dangerous proceeding unless very carefully controlled. If the titrations are made with the amount of patient's serum added to the complement and amboceptor the fallacy residing in the possible anticomplementary

PLATE I

Fig. 1.—Titration of Complement. Each of the tubes contains the amount of complement marked above the tube. In the titration, as illustrated, hemolysis is complete in the fourth tube, containing 0.05 c.c. of complement. Hence 0.05 c.c. is the complement unit and twice this amount is used in the actual test, or 0.1 c.c.

The tenth tube is a control tube containing no complement and it should show complete inhibition of hemolysis, as illustrated.

Fig. 2.—Titration of Amboceptor Papers. Each of the first five tubes contains a piece of amboceptor paper of the size indicated above the tubes. In the titration illustrated the first tube showing complete hemolysis is the third tube, containing a piece of paper measuring 5 by 3 mm. Hence the unit of amboceptor paper is a piece measuring 5 by 3 mm., and twice this amount is used in the test, or a piece measuring 5 by 6 mm.

The sixth tube is a control tube containing no amboceptor paper and should show complete inhibition of hemolysis, as illustrated.

Fig. 3.—Titration of Antigen. Tubes 1, 2, 3, and 4 contain respectively 0.05, 0.1, 0.15, and 0.2 c.c. of a 1 in 10 dilution of the antigenic extract, with 0.1 c.c. of a known syphilitic serum. Tube 5 contains 0.1 c.c. of the syphilitic serum without antigen. Tube 6 contains 0.1 c.c. of a normal blood serum with 0.2 c.c. of the diluted antigen. Tube 7 contains 0.1 c.c. of the normal serum without antigen. Tube 8 contains complement without amboceptor, while Tube 9 contains the hemolytic system, acting as a control of the latter.

A good antigen should give the results illustrated in this titration, causing complete inhibition of hemolysis in the presence of 0.05 c.c. of a syphilitic serum, as shown in Tube 1, and no inhibition of hemolysis with a normal serum in amounts as large as 0.2 c.c., as shown in Tube 6. Complete inhibition of hemolysis should occur in Tube 8 and complete hemolysis in 5, 6, 7, and 9.

Fig. 1. Titration of Complement.

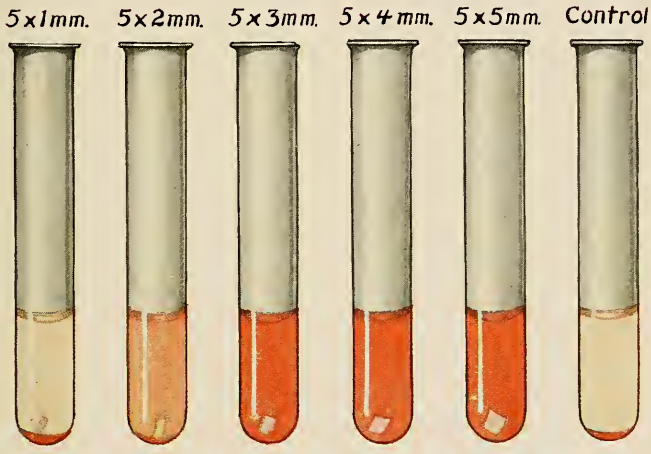

Fig. 2. Titration of Amboceptor Papers.

Fig. 3. Titration of Antigen.

action of the blood serum would be overcome, but this is a cumbersome procedure, especially when a large number of tests are to be made, and, in addition, there is still the factor of the natural inhibition of hemolysis which is present in almost all antigens. It is, therefore, much better to use an excess of complement in making the tests and it has been found perfectly safe in practice to double the unit of complement found by titration, when actually doing the test. In other words, *two* units of complement are used for each tube employed in the test.

In order to titrate the complement it is necessary that a standard amount of amboceptor and a standard suspension of human red blood corpuscles be adopted and strictly adhered to, although the strength of the blood suspension may be varied, provided the complement be titrated with it before each series of tests. Standard doses of amboceptor are adopted because this reagent changes little in strength during long periods of time, while if the amboceptor were to be titrated before each series of tests, a standard dose of complement would have to be adopted and the latter varies so greatly in strength that it is preferable to titrate the complement with a standard dose of amboceptor.

In the titration of the complement *two* units of amboceptor, as determined by titration, are employed, or the amount of amboceptor that is actually used in the test. The complement, amboceptor, and blood cells should be all added at once, for if the amboceptor and the blood cells are left in contact, without the complement, for any length of time, the cells will become "sensitized" and a smaller amount of complement will produce hemolysis.

In making the titration of the complement, either a 5 or 10 per cent suspension of human erythrocytes, in normal salt solution, may be used, but the writer prefers the 5 per cent suspension as it is more economic of amboceptor and is of sufficient strength to render the readings of the reaction easy. It should be remembered that the stronger the

blood suspension is the greater will be the amount of amboceptor and complement necessary to produce hemolysis, and the more extravagant the expenditure of these reagents, so that it is foolish to employ a stronger blood suspension for the test than is necessary to enable one to readily read the reactions, as the hemolytic system is merely used as an indicator of the presence in the patient's blood of the substances that absorb or bind complement.

In titrating the complement, test tubes measuring 100 by 12.5 millimeters are used. These tubes should be perfectly clean and, while they do not need to be sterile, the best technic is to have them sterilized. The complement is diluted with an equal amount of normal salt solution (0.85%) and graduated amounts are added to tubes containing the blood suspension and amboceptor, as illustrated in Table XVI.

TABLE XVI

TITRATION OF THE COMPLEMENT

TUBE NO.	AMOUNT OF SALT SOLUTION C.C.	AMOUNT OF BLOOD SUSPENSION, 5% C.C.	NUMBER OF AMBOCEPTOR UNITS	AMOUNT OF COMPLEMENT C.C.	
1	0.9	0.1	2	0.02	Incubate in water-bath at 37° C. for one hour and read. The tube showing complete hemolysis contains one unit of complement. Two units are used in making the test.
2	0.9	0.1	2	0.03	
3	0.9	0.1	2	0.04	
4	0.9	0.1	2	0.05	
5	0.9	0.1	2	0.06	
6	0.9	0.1	2	0.07	
7	0.9	0.1	2	0.08	
8	0.9	0.1	2	0.09	
9	0.9	0.1	2	0.10	
10	0.9	0.1	0	0.10	

After incubating in the water-bath at 37° C. for one hour, or in the ordinary bacteriological incubator, for two hours at the same temperature, the titration is read, and the tube that contains the smallest amount of complement which shows complete hemolysis is noted and this amount is called *one* unit of complement. For instance, if it is found that Tube No. 4, containing 0.05 c.c. of complement,

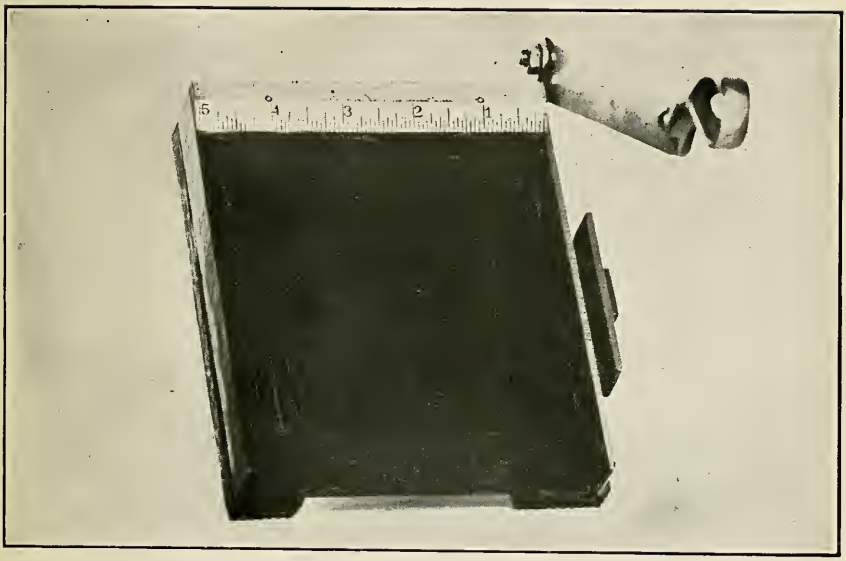

Fig. 8.

Amboceptor cutter. Front view showing gauge. Devised by Lieut. Colonel E. B. Vedder, Medical Corps, U. S. Army, for cutting amboceptor paper. (From Bulletin No. 8, War Department, Washington, D. C., Office of the Surgeon General, U. S. Army, 1915.)

is the first of the tubes to show complete hemolysis, the tubes preceding it showing only partial hemolysis, then 0.05 c.c. of complement constitutes one unit of complement, and in performing the actual test twice this amount or 0.1 c.c. will be used for the complement dose for each tube, or *two* units of complement. The amount of complement is doubled in order to provide for the anticomplementary action of the patient's blood serum and of the antigen, and has been found in practice to be sufficient to answer this purpose and at the same time not to cause fallacies in the test.

In reading the titration if there is the slightest cloudiness in any of the tubes hemolysis can not be said to be complete and in selecting the tube which represents one complement unit, the first that shows an absolutely crystal clear solution should be noted. The control tube, Tube No. 10, which contains no amboceptor, should show complete inhibition of hemolysis. If it does not, there is something wrong with the complement or the salt solution used in making the titration, and the titration should be repeated with new reagents.

This Titration of the Complement Must be Made Before Each Series of Tests or Very Serious Errors Will Develop.—The blood serum of guinea pigs varies greatly in complementary strength, so that it is excessively dangerous to use a stated amount of the serum for complement. A common observation, upon titrating different samples of guinea pig serum, is to find differences in complementary strength all the way from a unit equalling 0.03 c.c. to one of 0.08 c.c. and sometimes a serum will be found much stronger or weaker than these limits, so that the danger of using any definite amount is apparent. *The complement must be carefully titrated before each series of tests.*

Titration of the Hemolytic Amboceptor.—As already stated, the hemolytic serum or amboceptor used in the Wassermann test as recommended by the writer is prepared by immunizing rabbits to human red blood corpus-

cles. The human hemolytic system, as it is called, was adopted in order to obviate the danger arising from the natural antisheep amboceptor present in human blood serum, and which is frequently present in quantities sufficient to render a positive syphilitic serum negative unless special precautions are taken to guard against such an occurrence. While this result is not frequent in practice, when the sheep hemolytic system is used, the fact that it may occur, and that, in order to be perfectly safe, it is necessary to remove from each patient's blood the antisheep amboceptor that may be present, renders the use of the human hemolytic system preferable, both from a scientific and practical point of view, and it is the system the writer has always urged for complement-fixation tests upon human blood serum.

The hemolytic amboceptor, having been prepared by impregnating suitable filter paper with it, as already described, must be titrated in order to determine the amboceptor unit. After the paper is thoroughly dry a strip is cut from one of the sheets measuring 5 millimeters in width and varying lengths of this strip are used in the titration, as shown in Table XVII. The outer edge of the saturated sheet of filter paper should be trimmed off before cutting the strip referred to, in order to avoid any concentration of the serum which might occur at that portion of the paper.

TABLE XVII

TITRATION OF AMBOCEPTOR PAPER*

TUBE NO.	AMOUNT OF SALT SOLUTION C.C.	AMOUNT OF COMPLEMENT UNITS	AMOUNT OF BLOOD SUSPENSION C.C.	AMOUNT OF AMBOCEPTOR PAPER MM.	
1	0.9	1	0.1	5 by 1	Incubate in water-bath at 37° C. for one hour or in incubator for two hours at same temperature, and read results.
2	0.9	1	0.1	5 by 2	
3	0.9	1	0.1	5 by 3	
4	0.9	1	0.1	5 by 4	
5	0.9	1	0.1	5 by 5	
6	0.9	1	0.1	None	

*If the unit of complement has not been determined against a known amboceptor by titration, use 0.05 c.c. of a 1:1 dilution of the complement as the unit. It may be necessary to carry the titration on further by using larger amounts of the amboceptor paper, but a good paper should give hemolysis in a piece 5 by 5 millimeters.

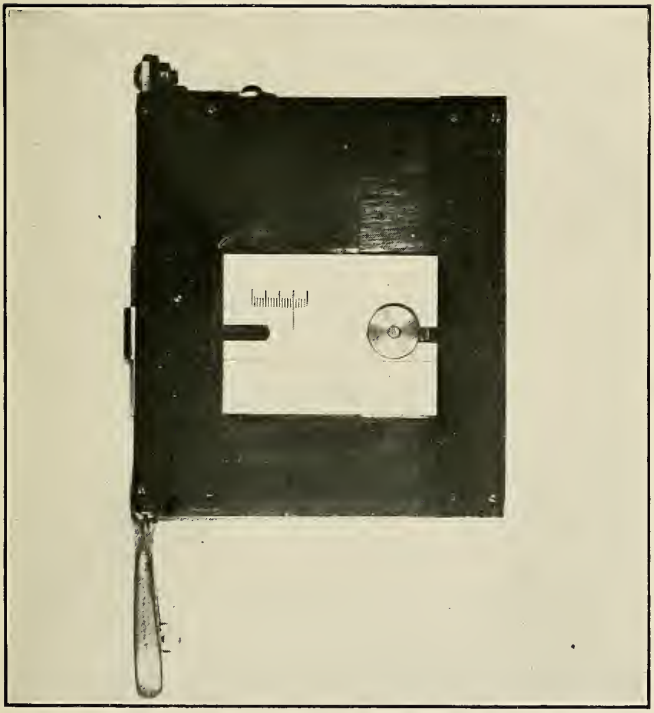

Fig. 9.

Amboceptor cutter. Rear view, showing scale and set screw. Devised by Lieut. Colonel E. B. Vedder, Medical Corps, U. S. Army. (From Bulletin No. 8, War Department, Office of the Surgeon General, U. S. Army, Washington, D. C.)

After incubating in the water-bath at 37° C. for one hour, or in the incubator, at the same temperature, for two hours, *the tubes being shaken every fifteen minutes in order to facilitate the liberation of the amboceptor serum,* the titration is read and the first tube to show complete hemolysis is noted and the amount of the paper contained in that tube is called *one amboceptor unit.* A good amboceptor paper should show complete hemolysis in tubes Nos. 2 or 3, or with pieces of paper measuring 5 by 2 or 3 millimeters, but paper measuring 5 by 5 millimeters can be used in the test. In many instances, the first tube will show complete hemolysis and, when this occurs, the paper may be reduced in width, or the strength of the blood suspension may be increased, a ten per cent suspension being used instead of a five per cent suspension. The control tube, No. 6, should not show any hemolysis and if it does it indicates that something is wrong with the blood suspension or the complement, and the titration will have to be repeated with new reagents.

In making the actual Wassermann test *two units of amboceptor paper* should be used in order to allow for the anticomplementary qualities of human blood serum and of the antigen. Thus, if one unit of the paper was found to be a piece measuring 5 by 2 mm. a piece 5 by 4 mm. should be used in making the test.

Amboceptor paper, being very stable, does not need to be titrated very frequently, once a month being amply sufficient, and such paper will keep, with little loss of strength, for several months.

Titration of the Syphilitic Antigen.—The antigens used in the Wassermann test must be carefully titrated and such titrations should be made at frequent intervals, as all antigens used in the test lose strength with more or less rapidity. To be on the safe side, the antigen should be titrated at least once in two weeks unless it be found that the particular antigens that are employed are very stable, when monthly titrations may be sufficient. A really good

antigen loses strength slowly and the writer has often worked with extracts that were practically as strong in antigenic qualities after several months as when first prepared, but the reverse is often true, and an antigen will be found that rapidly loses strength, thus making frequent titrations advisable. If possible, it is best to titrate the antigen before each series of tests.

An antigen must be titrated with a known syphilitic serum in order to determine its antigenic qualities; with a normal serum in order to demonstrate that it will not inhibit hemolysis when used with such a serum; and in the absence of either a syphilitic or normal serum, in order to determine whether or not it is anticomplementary or hemolytic of itself. Before titrating, the antigen should be properly diluted, which is accomplished by adding one part of the extract to nine parts of normal salt solution (0.85%). Any antigenic extract prepared in the manner recommended by the writer and diluted so as to produce an emulsion containing one part of the extract to nine parts of salt solution, that is neither anticomplementary nor hemolytic of itself or in the presence of a normal serum in a dose of 0.2 c.c. and that gives inhibition of hemolysis in the presence of a syphilitic serum in a dose of 0.05 or 0.1 c.c., will be found satisfactory in practice.

TABLE XVIII

TITRATION OF ANTIGEN FOR HEMOLYTIC PROPERTIES

TUBE NO.	AMOUNT OF SALT SOLUTION C.C.	UNITS OF COMPLEMENT	AMOUNT OF ANTIGEN EMULSION C.C.	AMOUNT OF BLOOD SUSPENSION. 5% C.C.	
1	0.9	2	0.05	0.1	Incubate in water-bath at 37° C. for one hour or in incubator for two hours, and read results.
2	0.9	2	0.10	0.1	
3	0.9	2	0.15	0.1	
4	0.9	2	0.20	0.1	
5	0.9	2	None	0.1	

Table XVIII illustrates the titration of antigenic extracts for the purpose of determining their hemolytic properties. The quantities of antigenic solution tested

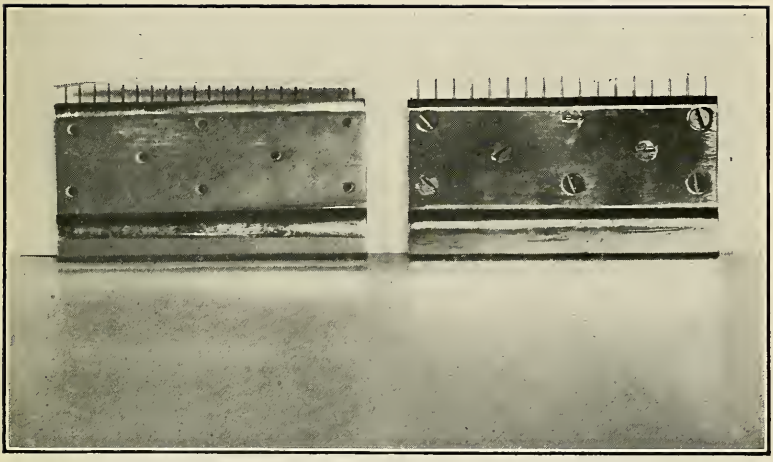

Fig. 10.

Amboceptor markers. Devised by Lieut. Colonel E. B. Vedder, Medical Corps, U. S. Army, for accurately measuring units of amboceptor paper. (From Bulletin No. 8, War Department, Office of the Surgeon General, U. S. Army, Washington, D. C.)

have been determined by a large number of experimental tests to yield satisfactory results.

In this titration none of the tubes should show hemolysis. If hemolysis occurs in all, including Tube 5, the control tube without antigen, it would demonstrate that hemolytic substances were present either in the complement or blood suspension, or both; but if hemolysis should occur in one or more of the tubes containing the antigen, the control tube remaining unhemolyzed, it would demonstrate that the antigenic extract is hemolytic; and, in this event, it should be discarded unless, upon being further diluted with salt solution, it is found to give satisfactory results. If none of the tubes containing antigen show hemolysis, the antigen is not hemolytic, and therefore, so far as hemolytic properties are concerned, it is safe to use in making the Wassermann test.

In order to determine whether *antigens are anticomplementary or not,* they are titrated as shown in Table XIX.

TABLE XIX

TITRATION OF ANTIGEN FOR ANTICOMPLEMENTARY PROPERTIES

TUBE NO.	AMOUNT OF SALT SOLUTION C.C.	UNITS OF COMPLEMENT	AMOUNT OF ANTIGENIC EMULSION C.C.	AMOUNT OF BLOOD SUSPENSION, 5% C.C.	UNITS OF AMBOCEPTOR PAPER	Incubate in water-bath at 37° C. for one hour or in incubator for two hours at same temperature and read results.
1	0.9	2	0.05	0.1	2	
2	0.9	2	0.10	0.1	2	
3	0.9	2	0.15	0.1	2	
4	0.9	2	0.20	0.1	2	
5	0.9	2	None	0.1	2	

As the result of this titration there should be complete hemolysis in all of the tubes. If Tube 5, the control tube, should show inhibition of hemolysis, it would prove that either the complement, amboceptor, or blood suspension, was inhibitory, but if any of the tubes containing antigen show inhibition, the control tube being hemolyzed, it would demonstrate that the antigen is anticomplementary, and, unless further dilution will remove this property without

injuring its antigenic value, the extract should not be used for the test.

Having tested an antigen for its hemolytic and anticomplementary properties, it is now necessary to titrate it against a known syphilitic blood serum to determine its antigenic value, and with a known normal blood serum to demonstrate that it will not give a positive reaction with nonsyphilitic sera. This titration is illustrated in Table XX.

As the result of this titration all of the tubes containing antigen should show complete inhibition of hemolysis in which syphilitic blood serum is also present and complete hemolysis in the tubes in which normal blood serum is present. Thus Tubes 1, 2, 3, and 4 should show absolute inhibition of hemolysis, while Tube 6 should show complete hemolysis. If Tube 6 shows any trace of inhibition, the antigen is not suitable and should be discarded; and the same is true if there is less than complete inhibition of hemolysis in Tube 2. There may be a slight trace of hemolysis in Tube 1 containing only 0.05 c.c. of the antigenic emulsion, without harm; but it will always be found better in practice to use only those antigenic extracts that give complete inhibition of hemolysis with 0.05 of the diluted antigen. Tube 7, which is a control of the normal serum used, should show complete hemolysis, and if it does not, another serum should be tested. Tube 8, the control of the complement, should show complete inhibition of hemolysis; and Tube 9, the control of the hemolytic system, should show complete hemolysis.

If these results are obtained, they demonstrate that the antigen, as diluted, is suitable for use in the Wassermann test as recommended by the writer, in that it produces inhibition of hemolysis in the presence of syphilitic blood serum in a dose as small as 0.05 c.c., and that it does not inhibit hemolysis in the presence of a normal serum in four times that dose, or 0.2 c.c. Such an antigen will give excellent results and even one that gives inhibition of hemolysis

TABLE XX

TITRATION OF ANTIGEN TO DETERMINE ANTIGENIC PROPERTIES

TUBE NO.	AMOUNT OF SALT SOLUTION C.C.	AMOUNT OF LUETIC SERUM INACTIVATED C.C.	UNITS OF COMPLEMENT	AMOUNT OF ANTIGENIC EMULSION C.C.		AMOUNT OF BLOOD SUSPENSION, 5% C.C.	UNITS OF AMBOCEPTOR PAPER	
1	0.9	0.1	2	0.05		0.1	2	
2	0.9	0.1	2	0.10		0.1	2	
3	0.9	0.1	2	0.15	Incubate in water-bath at 37° C. for one-half hour or in incubator for one hour.	0.1	2	Incubate in water-bath at 37° C. for one hour or in incubator for two hours at same temperature and read results.
4	0.9	0.1	2	0.20		0.1	2	
5	0.9	0.1	2	None		0.1	2	
6	0.9	Normal serum 0.1	2	0.20		0.1	2	
7	0.9	Normal serum 0.1	2	None		0.1	2	
8	0.9	None	2	None		0.1	None	
9	0.9	None	2	None		0.1	2	

with a syphilitic blood serum in a dose of 0.1 will be found satisfactory in practice, provided it is otherwise all right.

The amount of antigenic emulsion necessary to produce complete inhibition of hemolysis in the presence of a syphilitic blood serum is known as an *antigen unit. The antigen unit is not doubled in making the test as is the unit of complement and amboceptor.*

All blood sera used in titrating the antigen should be inactivated by heating for one-half hour at 56° C. and a neglect of this will produce most erroneous results.

Antigens should be kept in the ice box when not in use as exposure to room temperature for any length of time will result in their rapidly losing strength.

CHAPTER V

THE TECHNIC OF THE WRITER'S MODIFICATION OF THE WASSERMANN TEST. THE TEST UPON THE CEREBROSPINAL FLUID. THE ORIGINAL WASSERMANN TECHNIC. OTHER MODIFICATIONS OF THE WASSERMANN TEST.

The technic of the writer's modification of the Wassermann test was first fully published in 1913, in Bulletin No. 3, War Department, Office of the Surgeon General, entitled Studies in Syphilis, by Charles F. Craig, Captain, Medical Corps, U. S. Army and Henry J. Nichols, Captain, Medical Corps, U. S. A. The method has been used by the writer since 1910 with success and since 1913 it has been the standard method adopted in nearly all of the large army laboratories, and has been recommended for use in all army laboratories by Lieut. Col. Vedder, of the Medical Corps, who has devised special apparatus for use with the method. It is a modification of both the original Wassermann method and of that of Noguchi, following Wassermann in using an extract of foetal syphilitic liver as one antigen and in inactivating the patient's blood serum, and Noguchi in using a human hemolytic system instead of the sheep system.

This modification of the Wassermann test has been so thoroughly tried in the army, and so many thousands of tests have been made with it, that there is no question of its accuracy and value in the diagnosis of syphilis. Many different laboratory workers have used it and their results have all been in agreement as to its accuracy and

as, in army practice, we make many repeated examinations of the same case in order to control the treatment, any irregularities in the results of the test would have been soon discovered. In the hands of different workers the results have been almost identical with the same character of cases, as is instanced by Vedder,[49] and it is believed that it is as well adapted to civilian laboratories as to those of the army and will be found at once simple and accurate.

The method of preparing the reagents used in the test has already been described, as well as their titration, and in this chapter will be considered the exact technic of the test using the reagents as prepared and titrated.

The *glassware* used in preparing the various reagents for the test and in the test should be perfectly clean. No chemicals should be used in cleaning the glassware but only hot water and soap, followed by a thorough rinsing with hot water. All glassware, except the test tubes used in the test, should be sterilized by dry heat and one should be careful that a temperature of 180° C. is not exceeded, as a higher temperature than this is apt to so heat the ends of the small 1 c. c. pipettes as to close them. The test tubes do not need to be sterilized, and after washing, should not be plugged, but placed open end downward in a test tube basket and covered with a clean towel when not in use.

The pipettes used for measuring the patient's serum should be graduated in tenths and do not need to be graduated in hundredths, thus saving considerable expense where many tests are being done, as an individual pipette should be used with each patient's blood serum. At least a dozen 1 c.c. pipettes, graduated plainly in hundredths, the graduations being at such a distance apart that 0.02 c.c. can be accurately measured, should be in stock, for titration work and in making dilutions. In using any pipette graduated to the tip the last unit of graduation should not be used as it is very apt to be inaccurate. Great care should be exercised in using the pipettes to avoid

breakage, especially of the tip, and they should not be allowed to remain unwashed after using but should be immediately cleaned, as otherwise the blood serum will dry in them and will be found very difficult to remove owing to the small caliber of the pipette.

Collection and Preparation of the Patient's Blood Serum.—When specimens of blood for the Wassermann test are to be sent through the mail or kept for more than two days before they are tested, the blood should be collected with aseptic precautions and placed in sterile glass containers. If the test is to be made at once or within a day or two such precautions are not necessary but it is always essential that an aseptic technic be employed in collecting the blood, in order to avoid any danger of infection to the patient. The reason for observing these precautions has been noted in the discussion of the effect of contaminating bacteria upon the result of the Wassermann test. All specimens of blood serum for the test should be kept in the ice box until used, as exposure to room temperature may cause the serum to become anticomplementary.

Before taking the blood from the patient he should be questioned regarding the use of alcohol and if he states that he has used alcoholics within thirty-six hours the blood should not be taken but the patient told to return upon the following day, meanwhile abstaining from the use of alcohol in any form. This precaution is necessary owing to the fact that alcohol is capable of rendering a positive reaction negative, as shown by the writer and Nichols and already discussed.

Patients undergoing treatment for syphilis frequently give a negative Wassermann reaction during the time treatment is being administered, so that it is better to test the blood serum after treatment has been stopped for a period of two or three weeks. Blood for the Wassermann test should not be taken during a malarial paroxysm, as sometimes a positive reaction is given in such instances, nor should it be collected immediately after anesthesia or after

eating. Bile stained blood sera may inhibit hemolysis and should not be tested if it is possible to secure a specimen from the patient that is not bile stained. If a positive reaction occurs with a bile stained serum it should be remembered that it may be a false positive and the diagnosis of syphilis should only be made provisionally.

Blood serum should not be over five or six days old, at the most, when tested, as older sera frequently are anticomplementary. If a serum inhibits hemolysis in both the antigen and control tube another specimen should be requested. Inactivation often removes the anticomplementary properties of old sera but it is best not to employ them in the test if it is possible to avoid doing so.

Collection of the Blood.—The amount of blood serum required in the writer's modification of the Wassermann test does not exceed 0.2 c.c. so that 2 c.c. of blood is amply sufficient for the test. This amount may be easily collected from a puncture with a needle or glass needle just above the finger nail upon the dorsal surface of the middle finger in the adult, allowing the blood to drop into a small, sterile and perfectly dry glass vial until from one and one-half to two cubic centimeters are collected, or the blood may be collected from a puncture in the lobe of the ear with a Wright tube. Any form of special apparatus that has been devised for collecting blood for the Wassermann test is unnecessary and if venipuncture is objected to by the patient enough blood can easily be obtained as described above.

In the writer's opinion, the simplest and best method of obtaining blood for the test is to remove from 2 to 5 c.c. of blood from one of the large veins in the forearm or at the bend of the elbow with a sterilized glass syringe. The arm should be cleaned, the site of the puncture of the vein brushed with iodine, and the needle, held almost parallel with the surface of the arm, pushed through the skin and directly into the vein. The blood should be withdrawn slowly or the syringe can be dispensed with entirely and

the blood allowed to flow directly into a container from the needle. The operation is practically painless and with a little practice anyone can become very expert in the procedure. The advantage of the method is that more blood can be collected, thus insuring an abundant supply of the serum to be tested.

After collection, the blood is placed in a suitable glass vial, the serum allowed to separate, after which it is pipetted into a sterile glass vial or tube if it is to be mailed, or used at once in the test. The serum separates best if the blood be kept at room temperature for an hour and then placed in the ice box. All blood serum should be kept in the ice box until used, and when specimens are received by mail, they should be immediately placed in the ice box. A positive and negative blood serum should always be kept in stock for control sera and these should be kept in the ice box when not in use. All blood sera used for the Wassermann test should be clear in appearance and with no disagreeable odor. Milky or cloudy sera are often due to chyle but it is best, if possible, not to use such sera, although they may not cause inhibition of hemolysis.

Inactivation of the Blood Serum.—*All blood sera used in the Wassermann test as recommended by the writer must be inactivated by heating at 56° C. for one-half hour.* This is done for the purpose of removing the native complement in the blood serum and also for removing any thermolabile anticomplementary substances that may have developed through age or bacterial contamination. While the inactivation of the serum also tends to make it weaker in substances that give a positive reaction with syphilitic serum, as so well shown by Noguchi,[50] it is *absolutely necessary that inactivation be employed in any modification of the Wassermann test that employs either aqueous or alcoholic extracts of tissues which contain proteins* and this is true of the original Wassermann test and most of its modifications. When Noguchi's acetone insoluble antigenic extract is used the blood serum does not require in-

activation and this is one of the advantages that its author claims for it. However, in practice, the amount of harm done by inactivation, in weakening the reacting power of the blood serum, is small when compared with the harm which would result in using active serum, and it is only in very early infections or in those which have received treatment that inactivation might so weaken the serum as to render it negative.

In inactivating the blood serum extreme care should be taken that 56° C. is not exceeded, for exposure of the serum to a temperature of 60° C. will practically destroy its power of reacting with syphilitic serum. The temperature may be allowed to stand at 55° C. with as good results as at 56° C., but the latter figure should never be exceeded.

At the same time that the sera to be tested are inactivated, a known syphilitic serum and a known normal serum are also inactivated, to serve as controls of the test. In addition, there is always provided a tube for the serum to be tested, in which no antigen is placed, *the control tube,* and this is chiefly for the purpose of determining that the serum tested is not anticomplementary of itself. *This control tube of each serum tested should never be omitted for if it is no dependence can be placed upon the results of the test.*

The Writer's Technic.—Having inactivated the sera to be tested and the positive and normal control, and being provided with the proper blood suspension, hemolytic amboceptor, and syphilitic antigens, the test can be proceeded with.

Before doing anything else, however, the complement must be titrated as already described and the exact complement unit determined, and this is doubled for each tube in making the test. This procedure should never be omitted under any consideration. Some authorities prefer to titrate the amboceptor instead of the complement, but as the

complement is the substance in the test that is most apt to vary in strength, it should be titrated in preference to the amboceptor. As a matter of fact, the titration of the complement with a fixed dose of amboceptor, also determined by previous titration, can not result in any fallacies in the test, as the amount of complement necessary to produce hemolysis with the dose of amboceptor used, when doubled, is always sufficient to give accurate results when used in the test.

It is recommended that two antigens be used in testing each serum, one, the alcoholic extract of foetal syphilitic liver; the other, the alcoholic extract of normal human heart muscle, to which 0.4 per cent of cholesterin has been added. If both antigens can not be obtained, good practical results can be obtained by using one, as it is seldom that a positive result is obtained with one antigen and not with the other.

If two antigens are used three test tubes are needed for each serum to be tested, while if only one antigen is used, two tubes will be sufficient. The test tubes should be placed in racks having a double row, an anterior and a posterior. When three tubes are used, two should be placed in the anterior row of the rack and one in the posterior, while if two tubes are used, one should be placed anteriorly and one posteriorly. The anterior tubes contain the patient's blood serum together with the syphilitic antigen, while the posterior contains the patient's blood serum without the antigen, this tube acting as a control of the serum tested. Besides the tubes for the patient's blood serum, there must be an equal number of tubes for a known syphilitic serum, the positive control; and for a known normal serum, the normal serum control.

In making the test proceed as follows: In all of the tubes enumerated place 0.9 c.c. of normal salt solution (0.85%). In Tube 1 and Tube 2, anterior, if two antigens are used, or in Tube 1, if only one antigen is used, place 0.1 c.c. of the inactivated blood serum of the patient to be

tested, and the same amount in Tube 1, posterior. The same amount of syphilitic and normal blood serum is placed in the control tubes mentioned and in the same manner. Thus, if two antigens are used, in Tubes 3 and 4 0.1 c.c of the known syphilitic serum is placed, and the same amount in Tube 3, posterior, while if only one antigen is used, 0.1 c.c. of the syphilitic serum is placed in Tube 2, anterior, and in Tube 2, posterior. There is now added to every tube used in the test *two* units of complement and to each anterior tube *one* unit of the antigen used. If two antigens are used, one unit of each should be placed in the anterior tubes, alternately.

The tubes are now placed in a water-bath at 37° C. for one half hour, or in an ordinary bacteriological incubator, at the same temperature, for one hour, and at the expiration of this time there is added to each tube 0.1 c.c. of the 5 per cent suspension of human red blood corpuscles and *two* units of the amboceptor paper. The tubes are again incubated in the water-bath at 37° C., this time for one hour, or in the incubator for two hours, being shaken every fifteen minutes in order to facilitate the liberation of the amboceptor serum from the filter paper. After this incubation the tubes are placed in an ice box for one or two hours and the results then read. *Neglect of shaking the tubes, as recommended, will oftentimes result in partial positive reactions in sera that should react negatively.*

If desired a hemolytic and antigen control tube may be included but this is not necessary in practice as the titrations of the complement will show if anything is wrong with the hemolytic system, and an antigen control is unnecessary unless it has been some time since the antigen was titrated.

Results of the Test.—If all of the reagents used in the test are working properly the results, after the time allowed in the ice box, should be as follows:

The anterior tube or tubes, containing the patient's blood

serum, and the antigen or antigens, should show complete inhibition of hemolysis (positive reaction), if syphilis is present; while the posterior tube, containing the patient's serum without antigen, should show complete hemolysis. Of course, various degrees of inhibition will be shown in the anterior tube or tubes if the case is an early one, treatment has been given, or if the reaction is weak. If the patient is not syphilitic there should be complete hemolysis in the anterior tube or tubes as well as in the posterior tube. The posterior, or control tube of the patient's serum, should always show complete hemolysis, and if it does not, another specimen should be requested.

The anterior tube, or tubes, containing the known syphilitic blood serum, or the positive control, should show complete inhibition of hemolysis (positive reaction) while the posterior tube of the same serum without antigen, should show complete hemolysis. If inhibition occurs in this tube it demonstrates that the control positive serum is worthless.

The anterior tube, or tubes, containing the known normal serum, or the normal control, with antigen, should show complete hemolysis, as should the posterior tube of this set.

If a control tube for the antigen and for the hemolytic system be used, both should show complete hemolysis.

The Test Upon the Cerebrospinal Fluid

The great value of the Wassermann test when performed with the cerebrospinal fluid in demonstrating the existence of syphilitic infection of the central nervous system renders it almost imperative that, before assuring a patient that he is free from infection, the spinal fluid be tested. Not only is this true, but the fluid should be tested in all cases early in the infection, if possible, in order to ascertain whether the nervous system is involved, and if this were more generally done, it is probable that many of

PLATE II

FIG. 1.—Reading of Results of Wassermann Test. The first tube shows complete inhibition of hemolysis and may be read four-plus or double-plus, according to the nomenclature used in the individual laboratory.

Tubes 2 and 3 show three-plus and two-plus reactions, or both are included and reported as a plus reaction.

Tubes 4 and 5 show single-plus and plus-minus reactions, or may both be included and reported as a plus-minus reaction.

Tube 6 shows a minus or negative reaction.

FIG. 2.—Wassermann Test. Positive Reaction. Tube 1 contains the patient's blood serum and syphilitic antigen and shows complete inhibition of hemolysis or a positive reaction. Tube 2 is a control of the patient's serum without antigen, and shows complete hemolysis. Tube 3 is a known syphilitic control serum. Tube 4 is a control of the positive serum, without antigen. Tube 5 is a known normal serum and Tube 6 a control of the normal serum without antigen.

FIG. 3.—Wassermann Test. Negative Reaction. Tube 1 contains the patient's blood serum and syphilitic antigen and shows complete hemolysis, or a negative reaction. Tube 2 is a control of this serum without antigen. Tube 3 is a known positive serum and shows complete inhibition of hemolysis. Tube 4 is a control of the known positive serum without antigen. Tube 5 is a known normal serum with the antigen and Tube 6 a control of the normal serum without antigen.

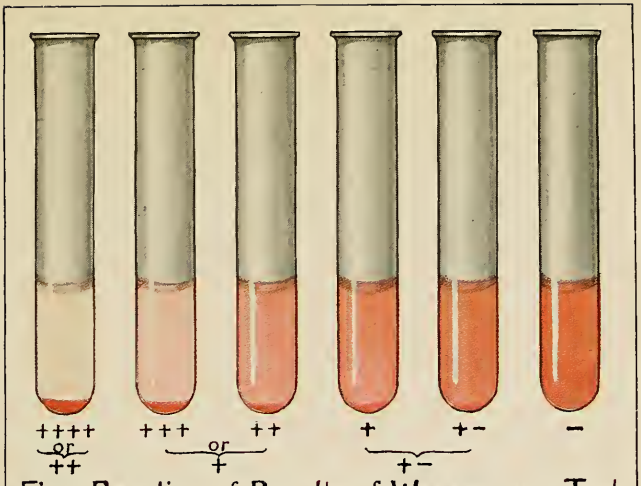

Fig.1 Reading of Results of Wassermann Test

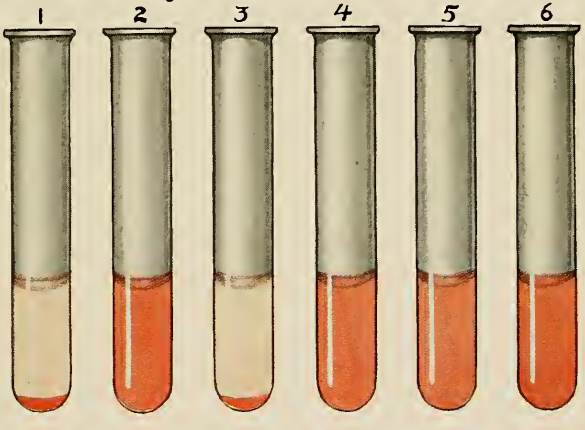

Fig.2 Wassermann Test Positive Reaction

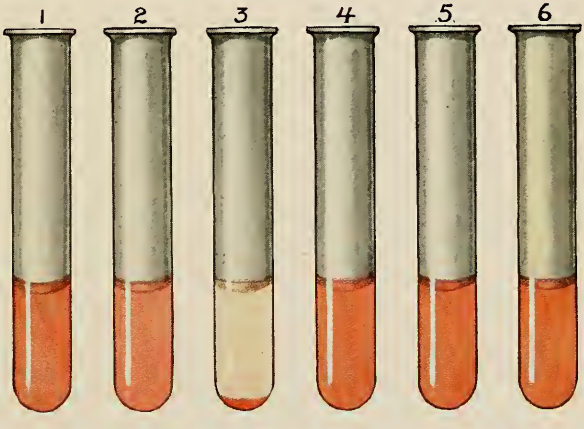

Fig.3 Wassermann Test Negative Reaction

Plate II.

the severe complications characteristic of involvement of the central nervous system could be avoided.

Collection of the Cerebrospinal Fluid.—The cerebrospinal fluid is collected by lumbar puncture and when properly performed, this operation is devoid of danger. The patient should be in bed and should remain in bed for twenty-four hours after the puncture. The writer is well aware of the fact that scores of punctures of the spinal canal are made in the physician's office, the patient afterward being allowed to walk home or to attend to his business, but, in his opinion, this practice is unjustifiable and should be discouraged, for while the danger of complications may be small, deaths have occurred during or shortly after the procedure when thus performed, and severe symptoms are not uncommon.

The lumbar region is washed carefully with soap and water, alcohol and ether, and the region over the lumbar vertebrae brushed with iodine solution. The site of puncture is most easily ascertained by running the finger along the spines of the vertebrae until the so-called "soft spot" is reached, which is indicated by a distinct feeling of softness to the finger and is situated between the third and fourth lumbar vertebrae. The patient may sit upon a stool with his back bent so as to bring the spinous processes of the vertebrae close to the surface, or lie upon the left side upon the edge of the bed. The latter posture is necessary in the case of the sick but the sitting posture is suitable for ordinary ambulant patients. The needle used in the puncture should be of flexible steel measuring 10 cm. long, with a bore of 1 to 1.5 mm., while for a child the needle should be shorter but of the same bore. The needle should be sterilized before being used, as well as the stylet which should accompany it.

The space between the third and fourth lumbar vertebra having been ascertained the puncture is made directly in the median line, which I have found much more satisfactory than the lateral puncture recommended by many writ-

ers. The needle is held firmly and the puncture made quickly, the direction being straight forward. Enough force should be used to push the needle quickly through the skin and muscles but as soon as the spinal ligaments are reached it may be pushed forward more slowly until there is a sudden sensation of loss of resistance when the needle is in the spinal canal. The stylet is now withdrawn and the spinal fluid, if the puncture is successful, will flow from the needle. If it does not flow, the needle may be shifted very gently or the patient told to take a deep breath, or the stylet may be replaced in the needle and gently pushed forward to clear the tube of any materials that have been carried into it by the influx of the spinal fluid. If none of these procedures are successful the puncture is said to be a "dry" one and there is little use in repeating it.

Not over 5 c.c. of fluid should be withdrawn, as that is amply sufficient for the test and the less fluid withdrawn the slighter will be the symptoms following the puncture. The fluid often comes from the needle under considerable pressure and this should be noted upon the slip sent to the laboratory with the fluid. It should be free from blood but can be used even if blood corpuscles are present, by centrifuging the fluid until they are deposited at the bottom of the tube and pipetting off the clear fluid.

The spinal fluid is kept in the ice box until used and should not be *inactivated, as is the blood serum, before it is tested.*

The same procedure is followed in testing spinal fluid as in testing blood serum, but different quantities of the fluid are tested, as in some infections of the central nervous system, as paresis, a very small amount of the fluid will give a positive reaction, while the same amount in cerebrospinal syphilis would be negative. Therefore it has always been the writer's practice to test 0.2, 0.5, 0.7 and 1 c.c. of the spinal fluid, thus making four tubes for the antigen tubes and four for the control amounts of fluid,

if only one antigen is used, and double this number if two antigens are used in the test.

Precautions to be Observed in Lumbar Puncture.—Patients who are very weak or debilitated should not have a lumbar puncture performed unless it is absolutely necessary. Those suffering from paresis and tabes bear puncture exceedingly well and seldom complain of pain, but nervous normal adults frequently speak of the pain of the puncture and of disagreeable symptoms following it. Headache may be severe if the fluid is withdrawn rapidly and if the bone is punctured or bruised by the needle during the procedure pain may last for several hours afterwards. Nausea is sometimes observed, especially if the fluid is rapidly withdrawn, so that it is always best to allow the fluid to flow as slowly as possible, thus relieving pressure gradually. Nonne[51] warns against lumbar puncture in cases of cerebral tumor, several deaths having followed the operation in this condition, and states that he has himself observed four cases of sudden death in such patients following the puncture.

The patient should remain in bed for twenty-four hours after the puncture, if possible, and neglect of this precaution often leads to disagreeable symptoms. In a case which was observed in the writer's practice, very severe headaches and attacks of dizziness occurred for several days after puncture because the patient insisted upon immediately returning to work. Diarrhea is a symptom sometimes observed, especially if the fluid has been withdrawn rapidly.

The Reading of the Reaction and Nomenclature Employed.—The results of the Wassermann test are variously reported from different laboratories and unless one is acquainted with the meaning of the various terms used, grave errors may result in the interpretation of the results of the test.

In perhaps the large majority of Wassermann labora-

tories the results of the test are recorded as four-plus (++++), indicating complete inhibition of hemolysis, and, therefore, a strongly positive reaction; three-plus (+++), indicating about 75 per cent inhibition of hemolysis, or a moderately positive reaction; two-plus (++), indicating 50 per cent inhibition of hemolysis, or a weakly positive reaction; plus (+), indicating from 25 to 50 per cent inhibition of hemolysis, or a doubtful reaction; plus-minus (+−), indicating less than 25 per cent inhibition of hemolysis or a doubtful reaction; and minus (−), indicating complete hemolysis, or a negative reaction.

In the army laboratories a more simple nomenclature has been employed for the reason that only absolute inhibition of hemolysis is considered a positive reaction, all other degrees of inhibition being considered doubtful. This standard has been adopted principally that the soldier may be given the benefit of any doubt that may exist regarding syphilitic infection. Thus four designations are used in the army laboratories, as follows: Double-plus (++), indicating complete inhibition of hemolysis, and, therefore, a positive reaction; plus (+), indicating anything between complete inhibition and 50 per cent of hemolysis; plus-minus (+−), indicating anything between 50 per cent of hemolysis and complete hemolysis; minus (−), indicating complete hemolysis, or a negative reaction.

Comparing this nomenclature with the foregoing, the three-plus and two-plus reactions would correspond to our plus reaction, the one-plus with our plus-minus reaction, and the four-plus with our double-plus reaction.

The writer believes that for use in civilian life the nomenclature of results first mentioned, i. e., four-plus, three-plus, two-plus, plus, plus-minus and negative, is the most useful; but anything below a three-plus reaction, in his opinion, should be considered doubtful, unless there is very good evidence of syphilitic infection, or the patient has received antisyphilitic treatment.

The Original Wassermann Technic

In the original Wassermann technic an aqueous extract of syphilitic liver was employed as the antigen; the blood sera were inactivated by heating them at 55° C. for one-half hour before testing; and a sheep hemolytic system was used. The *complement* was fresh guinea pig serum diluted 1:10 with normal salt solution, the dose being arbitrarily fixed at 1 c.c. of the diluted serum. The *blood suspension* was a 5 per cent suspension of sheep erythrocytes in normal salt solution, and the dose for each tube was 1 c.c. of the suspension. The *hemolytic amboceptor* was the blood serum of a rabbit immunized with sheep's erythrocytes and this was titrated carefully before each series of tests. The antigen, as stated, was an aqueous extract of foetal syphilitic liver, prepared in the manner already described and carefully titrated before using. Two tubes were used for each of the blood sera tested; two for the positive control; two for the normal control; and three tubes for control of the hemolytic system, antigen and blood suspension.

The tubes are arranged in double rows except the three control tubes. For each serum tested there should be an anterior and posterior tube and the same for the known positive serum and for the known normal serum. In each of these tubes there is placed 1 c.c. of the complement, diluted 1:10. In the anterior tube of the test set of two there should be placed 0.2 c.c. of the patient's inactivated blood serum, and the same amount in the posterior tube of this set. In the anterior tube and posterior tube of the positive control set there should be placed 0.2 c.c. of the known positive serum, and in the two tubes of the normal serum control there should be placed the same amount of a known negative blood serum. In the anterior tubes the proper dose of antigen is placed and enough normal salt solution to bring the total amount in the tubes up to 3 c.c. Of the three control tubes, the first is the *antigen control* and should contain the dose of antigen used, 1 c.c. of the com-

plement, and enough salt solution to equal 3 c.c.; the second is the *hemolytic system* control, and should contain 1 c.c. of the complement and 2 c.c. of the normal salt solution; while the third is the *blood suspension* control, and contains 1 c.c. of the corpuscle suspension and 2 c.c. of salt solution.

The tubes are shaken and incubated for one hour in the incubator and to each tube is then added *two* units of amboceptor serum, as determined by the titration and 1 c.c. of corpuscle suspension. The blood suspension control does not, of course, receive this suspension. The tubes are shaken and incubated until the blood serum controls (the posterior tubes) are hemolyzed, and the results read at once or after several hours in the ice box.

If the patient's serum is positive, the anterior tube will show complete or almost complete inhibition of hemolysis, while the posterior tube, containing the serum without antigen, should show complete hemolysis; if the patient's serum is negative, the anterior and posterior tubes should both show complete hemolysis. The anterior tube of the positive control set should show complete inhibition of hemolysis, while the posterior tube should show complete hemolysis; the anterior tube of the normal serum control and also the posterior should show complete hemolysis. The antigen control tube should show complete hemolysis, as should the hemolytic system control, while the blood suspension control should show complete inhibition of hemolysis.

The nomenclature used in reporting the test is that already described, but in reporting results to physicians it is better to report them simply as positive, very suspicious, suspicious, doubtful or negative.

Other Modifications of the Wassermann Test

There have been many modifications of the original Wassermann technic for the complement-fixation test in syphilis and the most important will be here considered.

Noguchi's Modification.—In Noguchi's modification a human hemolytic system is used, the serum to be tested is not inactivated, and the acetone insoluble lipoid antigen is employed, the preparation of which has already been described. If the blood serum can not be tested at once, inactivation should be employed but better results are obtained with active serum. Capillary pipettes are used in measuring the patient's serum, one drop of the active serum being employed, or practically 0.02 c.c. and four drops of the inactivated serum, or practically 0.08 c.c.

Noguchi thus describes the technic of the test:[52] A rack containing two rows of holes should be used, an anterior and a posterior row. "For each test two tubes are required, one in the front row and its control in the rear row. There will also be two pairs of tubes to serve as positive and negative controls.

"Put into each of two small test tubes front and rear one drop (0.02 c.c.) of the serum to be tested from a capillary pipette. When using inactivated serum put 4 drops (0.08 c.c.) into each tube. Use 0.2 c.c. of cerebrospinal fluid not inactivated. Add to each tube 0.1 c.c. of 40 per cent fresh guinea pig serum made by adding 1 part of complement to 1½ parts of 0.9 per cent salt solution. To the front tube add the unit of antigen determined by titration. Then to both tubes add 1 c.c. of the one per cent suspension of washed human corpuscles (the first procedure). In case of using the ten per cent corpuscle suspension add 0.9 c.c. of salt solution and two units of amboceptor (the second procedure). Shake the tubes thoroughly from time to time to distribute the reagents throughout the mixture.

"With every series of tests it is necessary to carry *two sets of controls* and for this purpose four additional tubes are necessary. To each of the first pair of these, one in the front and one in the rear row, one capillary drop of a syphilitic serum known to give a positive reaction is added. This will serve as a positive control. To the second pair

one drop of normal serum known to give a negative reaction should be added. This tube will serve as a negative control. Now put into each tube complement and into the tubes of the front row antigen, adding finally 1 c.c. of the one per cent corpuscle suspension (first procedure), or 0.9 c.c. of salt solution and 2 units of amboceptor (second procedure) to each tube.

"Place the rack holding these pairs of tubes in a water-bath, thermostat, or warm place not over 37° C. Allow an hour from the time the mixture is made for the antibody to combine with the antigen and for complement to be fixed. If a water-bath is used 30 minutes is a sufficient length of time. The contents of the tubes in the first procedure (first method) are as follows:

"Rear. Test serum plus complement (2 units) plus 1 c.c. 1 per cent corpuscle suspension.

"Front. Test serum plus complement (2 units) plus antigen plus 1 per cent corpuscle suspension.

"The contents of the tubes in the second procedure are as follows:

"Rear. Test serum plus 2 units complement plus 2 units amboceptor paper.

"Front. Test serum plus complement (2 units) plus antigen plus 2 units amboceptor.

"First incubation at 37° C. for 1 hour, then add to each tube of the first procedure a slip bearing two units of amboceptor, as follows:

"Rear. Above, plus amboceptor (2 units).

"Front. Above, plus amboceptor (2 units).

"To each tube of the second procedure 0.1 c.c. of the ten per cent corpuscle suspension, as follows:

"Rear. Above, plus 10% corpuscle suspension (.1 c.c.).

"Front. Above, plus 10% corpuscle suspension (0.1 c.c.).

"Allow two hours in the thermostat or one hour in the water-bath. After final incubation the tubes are kept at room temperature for a few hours before the results are recorded."

It will be noted that Noguchi gives two procedures or methods of making the test but the first procedure is to be preferred, in the opinion of the writer.

Table XII illustrates the method of performing Noguchi's modification of the Wassermann test by the first procedure recommended:

TABLE XXI

NOGUCHI'S METHOD OF PERFORMING THE WASSERMANN TEST WITH HIS MODIFICATION

SET FOR DIAGNOSIS	POSITIVE CONTROL SET	NEGATIVE CONTROL SET	
Posterior Row	*Posterior Row*	*Posterior Row*	Incubation at 37° C. for one hour.
Unknown serum, 1 drop (active) Complement, 2 units 1 c.c. 1% corpuscle suspension	Positive serum, 1 drop Complement, 2 units 1 c.c. 1% corpuscle suspension	Normal serum, 1 drop Complement, 2 units 1 c.c. 1% corpuscle suspension	
Anterior Row	*Anterior Row*	*Anterior Row*	Addition of 2 units of antihuman amboceptor to all tubes.
Unknown serum, 1 drop (active) Complement, 2 units 1 c.c. 1% corpuscle suspension Antigen, 1 unit	Positive serum, 1 drop Complement, 2 units 1 c.c. 1% corpuscle suspension Antigen, 1 unit	Normal serum, 1 drop Complement, 2 units 1 c.c. 1% corpuscle suspension Antigen, 1 unit	Incubation at 37° C. for two hours longer, then at room temperature. One hour in water-bath sufficient and one-half hour for first incubation.

Hecht-Weinberg's Modification.[53]—In this modification the natural amboceptor present in human blood serum to sheep's corpuscles is used for the amboceptor, and for complement, that present in the patient's blood serum is utilized. In other words, the serum to be tested furnishes both amboceptor and complement. Therefore, the blood serum must not be inactivated before use and should be used as soon as possible after the specimen is taken. The modification is claimed to give more delicate results than the usual Wassermann test but the writer does not believe that it should be used generally, owing to the errors that may occur through false positive reactions occurring with the usual antigens employed and because the amount of natural amboceptor to sheep erythrocytes is such a vari-

able quantity in human blood, being excessive in some instances and practically absent in others. The acetone insoluble antigen of Noguchi should be used with this modification.

Gradwohl's Modification of the Hecht-Weinberg Modification.—Gradwohl[54] has greatly improved the technic of the Hecht-Weinberg modification of the Wassermann test by ascertaining the hemolytic strength of each serum to be tested to sheep corpuscles; i. e., the hemolytic index of each serum to sheep corpuscles is worked out before the serum is finally tested. Gradwohl[55] thus describes his technic:

"Place in a rack fourteen small test tubes. The first ten of these tubes are used to determine the hemolytic index of the suspected blood. By this I mean the exact amount of hemolytic amboceptor present in the given blood serum. The last four tubes are used in the actual test. Add 0.1 c.c. of fresh, unheated, patient's blood serum to each of the first ten tubes. Then add decreasing amounts of normal salt solution to these tubes, beginning with 1 c.c., then 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 c.c. to the succeeding nine tubes. Next add increasing amounts of fresh 5 per cent suspension of sheep's corpuscles, starting with 0.1 c.c. and ending with 1 c.c. Place the rack in the water-bath for one-half hour. The tube which last shows complete hemolysis constitutes the 'hemolytic index;' if it is tube 4, the index is 4, because this tube had received 0.4 c.c. of sheep's corpuscles and therefore we have obtained an idea as to how much sheep's blood is to be added to the last four tubes. The first three of these tubes (11, 12, and 13) constitute the tubes for the actual test, while the last tube in the rack (14) serves as the serum control tube. Tubes 11, 12, and 13 receive, therefore, the patient's serum, the proper amount of sheep's corpuscles, dependent on hemolytic index, rising strengths of antigen, but no complement and no amboceptor. Tube 14 receives only sheep corpuscles, but no antigen."

"In my technic I use 0.1 c.c. of a diluted antigen, determined by titration, in tube 11, 0.15 c.c. antigen in tube 12, and 0.2 c.c. in tube 13. In order to equalize the volume of fluid in these tubes, I add 0.2 c.c. normal saline to tube 11, 0.15 c.c. to tube 12, 0.1 c.c. to tube 13, and 0.3 c.c. to tube 14. The tubes are then agitated and placed in the water-bath for half an hour. These last four tubes are filled at the time I make the additions to the first ten and are left with them in the water-bath for one-half hour for fixation of complement, the rack is then taken out and the hemolytic index computed. If the index is low, say from 1: 4, I add 0.1 c.c. of sheep's blood to the last four tubes. If the index is between 5 and 7, I use 0.15 c.c. sheep's blood to the last four tubes; if between 8 and 10, I add 0.2 c.c.; if the index is over 10, I rack up ten more tubes and repeat the titration of the natural complement and amboceptor, then I estimate that; if between 11 and 15, I use 0.25 c.c.; if between 15 and 18, I use 0.3 c.c.; and if between 18 and 20, I use 0.35 c.c. If the patient's serum has an index below 2, I regard the reaction of doubtful value. If it is above 2, I regard it as absolute. The reaction is read off exactly as in the Wassermann, that is, inhibition or non-inhibition of hemolysis. If the amount of complement or amboceptor is very low, we can add the proper amount of guinea pig's serum or rabbit's immune serum, ascertained by preliminary titration."

Gradwohl claims that this modification, in his hands, is superior to the Wassermann reaction, giving 15 to 20 per cent more positive reactions than the latter, but the writer believes that it is so cumbersome that it could not be used in laboratories where scores and, sometimes, hundreds of tests are being made each day, as in army practice, and that the fact that false positives have been repeatedly reported by different observers who have used the test militates greatly against its value. It is an excellent control

of the Wassermann test but should not replace it in general laboratory practice.

Kolmer's Modifications.—Four different methods for performing the Wassermann reaction are recommended by Kolmer.[56] The *first method* is exactly similar in technic to the original Wassermann, except that an alcoholic extract of syphilitic liver is used instead of an aqueous extract. In the *second method* the technic is the same except that three different antigens are used with each blood serum tested and the amounts of each reagent are reduced one-half. Kolmer strongly recommends this method as being "simple, accurate, and reliable." The three antigens used are: 1. A cholesterinized alcoholic extract of human heart; 2, alcoholic extract of syphilitic liver; 3, acetone insoluble lipoids. It will thus be noted that four test tubes are required for each serum tested, one as control, and three for the antigen tubes. As regards the results, Kolmer says:

"With strongly positive serums there is complete inhibition of hemolysis with all three antigens. * * * In from 15 to 20 per cent of cases the cholesterinized extract shows a 50 per cent or more inhibition of hemolysis, whereas with the other two antigens the reactions are negative. In our experience the majority of such serums were taken from patients giving a frank history of syphilis of many years' standing, and from known cases undergoing treatment, further therapy being indicated until the reaction finally becomes negative when cholesterinized extracts are used. In a small proportion of cases a feebly positive reaction of 25 per cent or even less of inhibition of hemolysis may be found with the cholesterinized extract alone. Many of these reactions occur with serums of treated cases of syphilis; on the other hand a similar reaction may occur with about 5 per cent of normal serums, so that if the history and clinical conditions are clearly negative, a slight degree of inhibition of hemolysis (5 to 10 per cent) with the choles-

terinized extract and marked hemolysis with the other two antigens may be interpreted as a negative reaction."

The writer has quoted Kolmer regarding his results with cholesterinized extracts for antigens because his experience has been exactly similar and for this reason the writer firmly believes in the value of such extracts in the diagnosis of syphilis with the Wassermann test and in the control of the treatment of the disease. Properly titrated and controlled, and with experience gained by using them, so that a proper interpretation may be placed upon the results obtained, cholesterinized antigenic extracts are perfectly safe and furnish a much greater amount of information regarding the existence of a syphilitic infection than do other antigenic extracts.

Kolmer's third method is devised in order to measure the amount of syphilitic antibody in the patient's blood serum. He employs only one antigen, either an alcoholic extract of syphilitic liver or the acetone insoluble lipoid antigen. The technic is about the same as in the original Wassermann but eight tubes are used for each patient's serum, increasing amounts of the serum, diluted 1:10 being placed in them, together with the unit of antigen determined by titration, only one antigen being employed. The *fourth* method is somewhat similar, but in this the amount of syphilitic antibody in the patient's blood serum is measured by the number of hemolytic doses of complement fixed by a constant amount of antigen.

As routine methods, where many tests are being performed, the *third* and *fourth* methods of Kolmer are not practical, as they consume too much time and material. The *second* method is an excellent one, but it appears to the writer that practically the same results are obtained by using two antigens, instead of three, as recommended in his modification. The writer also believes, as already stated, that a human hemolytic system is preferable to the sheep system, which is used in all of Kolmer's methods, owing to the possibility of the natural antisheep ambocep-

tor present in nearly all human blood serum interfering with the delicacy of the test.

Despite all the claims that have been made that the presence of antisheep amboceptor in human serum is a negligible factor in the Wassermann test, the fact remains that in some blood sera there is so much of this amboceptor present that no dependence can be placed upon the results of a Wassermann test with them unless it is first removed, and it is certainly logical to believe that, in some cases, this amboceptor when combined with the amboceptor added, would render a reaction negative that, with the human hemolytic system, would be positive. This factor would frequently be of very great importance in any method of performing the test in which titrations of the amount of syphilitic antibody in the patient's serum were used.

The modifications of Tschernogubow[57] which utilize the natural complement and amboceptor against guinea pig corpuscles present in human serum; of Detre and Brezovsky,[58] in which an antihorse hemolytic system is employed with rabbit complement; of Margarette Stern[59] who used fresh, active blood serum and the complement present in the blood serum tested; of Bauer,[60] who makes use of the antisheep amboceptor present in human blood serum; of Browning and McKenzie,[61] who use an antiox hemolytic system and a quantitative technic; and of Thompson,[62] who uses the complement present in the fresh blood serum of the patient, an antihuman hemolytic system, and the acetone insoluble lipoid antigen of Noguchi have all been recommended, from time to time, but none of them appear to be as reliable as the original Wassermann technic, the modifications recommended by Noguchi and Kolmer, or the writer's modification.

The Effect of Low Temperatures upon the Results of the Wassermann Test.—Quite recently several observers have called attention to the effect of incubating the com-

plement, antigen, and patient's blood serum at ice box temperature, claiming that more delicate results are obtained than when this incubation is made in the water-bath at 37° C. Zinsser[63] states that in his laboratory two series of tests were run, one with the first incubation at 37.5° C. for 30 minutes, the other with the first incubation in the ice box at 8 to 10° C., for three hours, the same blood sera being used in each series. The results showed 15 more positive reactions in the series incubated at ice box temperature, than in that incubated in the water-bath, and of these cases 7 were unquestionably syphilitic, two were treated syphilitics, and four were probably syphilitic.

In a recent valuable contribution, Smith and MacNeal[64] compared the results obtained with different antigens and different temperatures of incubation in the Wassermann test. They experimented with three antigens as follows: 1. A cholesterinized alcoholic extract of beef heart. 2. A plain alcoholic extract of beef heart. 3. An acetone insoluble extract of beef heart. Two series of tests were made, with the same blood sera, one in which the first incubation, with each antigen, was at 37° C. and one in which the first incubation was for 4 hours in the ice box at 8° C., with each antigen. Over 500 different sera were tested and their conclusions are important. They found that the cholesterinized antigen, with incubation at 8° C. for 4 hours proved a more sensitive test for syphilis than any of the other methods tried but that it was apt to give nonspecific complement fixation, and, therefore, if used alone it could not be depended upon in diagnosis. The simple extract antigen (alcoholic extract of beef heart), with the first incubation for 4 hours at 8° C. in the ice box, gave more sensitive results than the cholesterinized extract at 37° C. and in these experiments did not give any false positive results. The acetone insoluble extract was found less sensitive either at 37° C. or 8° C. than the cholesterinized extract and also less sensitive than the simple alcoholic extract at 8° C.

It was more sensitive than the simple extract at 37° C., and gave no false positive results in this series of tests. From their results it is evident that the cholesterinized extract at 8° C. gave the highest percentage of positive results, but sometimes gave a false positive reaction, while the simple alcoholic extract gave the best practical results, when the first incubation was made at 8° C., as it gave better results than the cholesterinized extract at 37° C., and did not give any false reactions.

The subject of the ice box temperature for the first incubation in the Wassermann test deserves further study, and, if these observations are confirmed, this temperature should be adopted in practice. If it is found reliable it will probably be best to use simple alcoholic extracts for antigen instead of cholesterinized extracts, but more work should be done upon the subject before the method is generally adopted.

The Titration of the Strength of the Wassermann Reaction.—In using the Wassermann test as a control of the efficiency of treatment, it is often advisable to test the strength of the reaction, from time to time, especially if sera show a double-plus (four-plus reaction) repeatedly, although intensive treatment is being administered. In such cases, the physician can not be sure that the treatment is having any effect unless the blood serum is titrated as to its fixing or absorbing power of complement in the presence of suitable antigen. This is done by diluting the blood serum in the following manner, as recommended by Vedder:[65] Six tubes are used in addition to the usual number used in the Wassermann test as recommended by the writer. In the first tube is placed 1.9 c.c. of normal salt solution, and in each of the other tubes 1 c.c. of normal salt solution. In the first tube is placed 0.1 c.c. of the serum to be titrated and the tube is thoroughly shaken. One cubic centimeter is taken from this tube and placed in the second tube, which is shaken, and the same amount is carried to the third tube, and the operation repeated

until the sixth tube is reached, from which, after it is shaken, 1 c.c. is discarded. After the dilutions are thus made each tube will contain 1 c.c. of fluid and the dilutions of the blood serum will be as follows: Tube 1, 0.05 c.c.; Tube 2, 0.025 c.c.; Tube 3, 0.012 c.c.; Tube 4, 0.006 c.c.; Tube 5, 0.003 c.c.; and Tube 6, 0.0015 c.c.

The test is then made in the usual manner with all of the dilutions and the results recorded as in Table XXII.

Table XXII

Results of Titration of Blood Serum of ————————

Date of Test	Dilutions						
	0.1 c.c.	0.05	0.025	0.012	0.006	0.003	0.0015
Previous Result. ++ with 0.05 c.c.	++	+	+−	−	−	−	−

This method of titrating the blood serum of patients under treatment is recommended in all cases in which persistent double-plus (four-plus) reactions are reported, as it is the only way in which the physician can be sure that the particular method of treatment being pursued is proving efficient. In many instances the titration will demonstrate that the smaller amounts of serum become progressively negative, thus proving the efficiency of the treatment, although the usual amount of serum tested, i. e., 0.1 c.c., may give a positive reaction.

CHAPTER VI

COMPLEMENT FIXATION IN SYPHILIS WITH ANTIGENS PREPARED FROM PURE CULTURES OF TREPONEMA PALLIDUM

The success attending Noguchi's efforts to obtain *Treponema pallidum* in pure culture lead to the hope that antigens prepared from such cultures would be effective in the Wassermann test and that in this manner a truly specific antigen would be obtained and the reaction become a true antigen-antibody reaction. It is obvious that if such a specific antigen could be obtained the value of the Wassermann test, great as it is at present, would become still greater, as the positive reactions frequently observed in yaws, tubercular leprosy, and with less frequency, in malaria and trypanosomiasis, would be eliminated. The subject has already been briefly discussed but it is thought of enough importance to consider somewhat more extensively in this chapter.

Schereschewsky, in 1909,[66] was the first to attempt to secure an antigen from cultures of *Treponema pallidum.* Unfortunately, his culture was an impure one and for this reason his results were not conclusive. He was able to obtain complement fixation with syphilitic serum, with his antigen, but also obtained positive results with some of the control antigens with which he worked. It is now generally admitted that he was not working with cultures of *Treponema pallidum* at all but with *Treponema microdentium,* and, as will be shown later, his positive results were in the nature of a group reaction, as the writer and Nichols have shown that this organism will react positively with syphilitic serum, if used as an antigen. In addition,

Schereschewsky's cultures were contaminated with bacteria and it is possible that some of his positive results were due to this fact, for, as already noted, certain bacteria, when growing in normal blood serum, produce substances which give a positive reaction with the Wassermann test, and it is not improbable that bacteria growing in the horse serum medium, which he used for his cultures, may have produced lipoidal substances which were capable of giving positive results. However, his work was very suggestive and, without doubt, stimulated research along these lines.

In 1912, Noguchi,[67] who had succeeded in isolating and growing *Treponema pallidum* in pure culture, published the results of his work upon complement fixation in syphilis with an antigen prepared from such cultures. His antigen was an aqueous extract of *Treponema pallidum*, and he also worked with an emulsion of the organisms obtained from the testicles of infected rabbits. His researches demonstrated beyond doubt that, in certain cases of syphilitic infection, it is possible to obtain complement fixation with antigens prepared from a pure culture of *Treponema pallidum* and that, in such instances, the reaction is indeed a true antigen-antibody reaction.

Noguchi found that, in cases of syphilis that had been treated or in which the disease was latent, he often obtained a positive reaction with the culture antigen when the ordinary antigens used in the Wassermann test gave a negative result, and that the culture antigen very rarely gave a positive result in primary and secondary cases of the disease. Therefore, he concludes that the reaction with the pallidum antigen may be regarded as an index of the resistance of the patient to the infection, stating "We have in the Wassermann reaction a fair measure of the activity of the infecting agent, and now we will have in the pallida fixation reaction a gauge for the defensive activity of the infected host."

Noguchi's conclusions regarding his work with the pallidum antigen are as follows:

1. The Wassermann reaction is caused by the lipotropic substances in sera but not by the antibodies which combine specifically with the pallida antigen.

2. The fixation produced by the culture pallida antigen with certain syphilitic sera is caused by the specific antibodies contained in the latter and may constitute a specific diagnostic method for syphilis.

3. The fixation caused by testicular extracts behaves like the culture pallida antigen in the majority of cases, but when the sera (syphilitic or leprous) contains abundant lipotropic substances, they may give a Wassermann reaction as well, which is not the case with the culture pallida antigen.

4. In the serum of rabbits with active syphilitic orchitis there is no indication of the presence of a sufficient amount of antibodies for the pallida antigen, although it gives a strong Wassermann reaction.

In 1912, the writer and Major Nichols published the results of some work upon specific antigens in complement fixation with syphilitic serum,[68] which differed somewhat from those of Noguchi, chiefly because of the nature of the antigen employed. In his work Noguchi used aqueous extracts of *Treponema pallidum,* while in our work alcoholic extracts were employed. Noguchi stated that it was impossible to extract the antigenic properties of the organism by alcohol and that only aqueous extracts contained them, but our results showed that alcoholic extracts were efficient, although they were not as efficient as aqueous extracts, and our work has since been confirmed by other authorities. It is true that alcoholic extracts owe a part of their activity to other substances than the specific complement-fixing substance of *Treponema pallidum* which is best extracted in water, but it is also true that the alcoholic extracts do contain this specific substance, as the results obtained can be explained in no other way, and differ so greatly from those obtained with ordinary alcoholic antigens used in the Wassermann test.

In our experiments we tested 51 syphilitic sera and 54 nonsyphilitic sera. Of the latter, 38 were from patients suffering from diseases other than syphilis and 16 were from normal individuals. Eleven rabbit sera were also tested. The sera were all inactivated by heating them at 55-56° C. for half an hour before testing.

The *stock antigen* used was an alcoholic extract of foetal syphilitic liver and the *culture antigens* were prepared from cultures kindly sent us by Dr. Noguchi. Alcoholic extracts were made of the cultures, filtered, the filtrate evaporated to one-third its original volume and titrated in a dilution of 1-10 with normal salt solution. Antigens were thus prepared from cultures of *Treponema pallidum, Treponema pertenue,* and *Treponema microdentium.* The test was performed in the same manner as that recommended in this work by the writer for the Wassermann test.

As a control of the culture antigens used an alcoholic extract of the medium employed in growing the treponemas was prepared in the same manner as the culture antigens.

Results with Normal Sera.—The blood serum of 16 normal individuals was tested with the stock antigen and with pallidum, pertenue, and microdentium antigen, and all gave a negative result. Not a trace of a reaction occurred in any of these tests so that it is evident that none of the antigens employed reacted with normal blood serum.

Results with Serum from Other Diseases than Syphilis.—The blood serum of 38 patients suffering from diseases other than syphilis was tested with the same antigens and was negative in every instance, except that a plus reaction was obtained with the microdentium antigen in a case diagnosed as arthritis. Syphilis could not be eliminated in this case but the stock syphilitic antigen gave a negative result with the serum.

Results with Serum from Syphilitic Patients.—The blood sera of 51 patients suffering from syphilis were tested and Table XXIII shows the results obtained. In recording

these tests the sign, ++, means absolute inhibition of hemolysis; the sign, +, at least 50 per cent of inhibition; and the sign, +−, weaker degrees of inhibition. The sign, −, indicates total lack of inhibition, while the sign 0 indicates that no test was made.

TABLE XXIII

COMPLEMENT FIXATION IN SYPHILIS WITH STOCK ANTIGEN AND SPECIFIC ANTIGENS IN FORTY INFECTIONS*

SERUM NO.	STOCK ANTIGEN	T. PALLIDUM ANTIGEN	T. PERTENUE ANTIGEN	T. MICRODENTIUM ANTIGEN	CONTROL ANTIGEN	REMARKS
1	++	++	0	0	−	Secondary, early
2	++	+	+−	0	−	Primary
3	++	+−	+−	0	−	Secondary
4	++	−	0	0	0	Latent, 18 years
5	++	+	+	+	−	Latent, 4 years
6	++	−	+−	−	−	Latent, 3 years
7	++	−	−	0	−	Tertiary, 10 years
8	++	−	0	0	−	Secondary, 9 months
9	+	+	0	0	0	Secondary, early
10	+	0	0	0	−	Primary
11	+	++	0	0	−	Primary, 2 weeks
12	+	+	0	0	0	Latent, 2 years
13	+	−	0	+−	0	Primary
14	+	−	0	+	−	Primary
15	+	+	0	+	−	Secondary, early
16	+	+	−	−	−	Secondary, 1 year
17	+	−	0	+	−	Tertiary
18	+	+	+−	+	−	Secondary
19	+	++	0	+	−	Tertiary
20	+	+−	+−	+−	−	Latent, duration unknown
21	+−	+−	0	0	−	Latent, 3 years
22	+−	+	0	0	−	Primary, 3 weeks
23	+−	+−	0	0	0	Secondary, early
24	+−	−	−	−	−	Primary, 2 weeks
25	+−	−	−	0	−	Epilepsy
26	+−	+−	0	+−	−	Arthritis, syphilitic
27	+−	+−	0	+−	0	Latent, 2 years
28	+−	+−	0	+−	−	Latent, 2 years
29	+−	+	0	+	−	Latent, 1 year
30	+−	+−	−	+−	−	Secondary, 1 year
31	+−	++	++	+	−	Latent, duration unknown
32	+−	+−	0	+	−	Latent, 3 years
33	+−	+−	+−	−	−	Secondary, 3 months
34	+−	+	+−	+−	−	Latent, 4 years
35	+−	+−	−	+−	−	Latent, 6 years
36	+−	+−	+−	−	−	Secondary, early
37	−	+−	−	+−	−	Latent, 2 years
38	−	+	+	+−	−	Latent, 1½ years
39	−	+−	−	−	−	Latent, 6 years
40	−	+−	−	0	0	Primary, 2 weeks

*Each serum tested was properly controlled as is usual in the Wassermann test.

It will be noted that no case reacted with the control culture medium antigen and that of the 36 cases giving any reaction at all with the stock extract antigen, no less than twenty-seven, or 75 per cent reacted also with the pallidum antigen, but the reactions were, as a rule, much weaker with the pallidum antigen than with the stock antigen. However, there were instances in which the pallidum antigen gave a stronger reaction than the stock antigen, as illustrated in serums 11 and 19, while in serum 38 the stock antigen gave a negative result and the pallidum antigen a plus reaction.

As regards the results given with the antigen prepared from the pure cultures of *Treponema pallidum,* 17 of the sera gave the same result as with the stock antigen, four gave weaker reactions, six gave stronger reactions, and four gave positive results in sera in which the stock antigen gave a negative result. However, the pallidum antigen gave seven negative reactions in twenty sera that gave absolute inhibition of hemolysis with the stock antigen, (not shown in table) thus proving that the pallidum antigen is untrustworthy in the diagnosis of syphilis.

Of the various syphilitic sera tested, fifteen were tested with all three antigens, and the results are valuable because they indicate the group nature of the complement fixation reaction obtained with these alcoholic extracts of *Treponema pallidum, Treponema pertenue,* and *Treponema microdentium.* Table XXIV gives the results obtained:

In four of the cases, the results were the same with all of the culture antigens but in the remaining cases they varied, the pallidum antigen giving results more like those of the stock antigen, while the pertenue and microdentium antigens gave quite similar results, although those of the microdentium approached nearer to those of the pallidum than did the pertenue.

Unlike Noguchi, we obtained positive results not only in latent syphilitic infections but also in primary and second-

TABLE XXIV

COMPLEMENT FIXATION IN SYPHILITIC SERUM WITH CULTURE ANTIGENS

SERUM NO.	STOCK ANTIGEN	T. PALLIDUM ANTIGEN	T. PERTENUE ANTIGEN	T. MICRODENTIUM ANTIGEN	CONTROL ANTIGEN	REMARKS
1	++	+	+	+	–	Latent, 4 years
2	++	–	–	–	–	Latent, 3 years
3	+	+–	+–	+–	–	Latent, duration?
4	+–	–	–	–	–	Primary, 2 weeks
5	+	+	–	–	–	Secondary, 1 year
6	+	+	+–	+	–	Secondary, duration?
7	+–	+–	–	+–	–	Latent, duration?
8	+–	++	++	+	–	Secondary, 3 months
9	+–	+–	+–	–	–	Latent, 4 years
10	+–	+	+–	+–	–	Latent, 6 years
11	+–	+–	–	+–	–	Latent, 2 years
12	–	+–	–	+–	–	Latent, 1½ years
13	–	+	+	+–	–	Latent, 6 years
14	–	+–	–	–	–	Primary, 2 weeks
15	–	+–	–	0	0	Secondary, 1 year

ary infections with the culture antigens. It should be remembered, in this connection, that Noguchi worked with an aqueous extract of *Treponema pallidum* while we used alcoholic extracts, and thus the variation may be explained, although the writer does not agree with Noguchi that it is impossible to extract from this organism specific complement-fixing substances with alcohol. Our results with these alcoholic extracts of treponemas are too much at variance with those obtained with the ordinary alcoholic extract of foetal syphilitic liver to make it at all probable that the entire complement-fixing or absorbing power of these culture antigens was entirely nonspecific in character, and our experiments with rabbit blood serum, which gave identical results with those of Noguchi, are evidence of this fact.

Results With Rabbit Blood Serum.—We tested the blood serum of two rabbits presenting acute symptoms of syphilitic infection; of three that had recovered from a syphilitic orchitis; of two that had recovered from yaws; and of four normal rabbits, and the results are given in Table XXV.

The important fact shown by these experiments is that

TABLE XXV

COMPLEMENT FIXATION WITH SPECIFIC TREPONEMA ANTIGENS IN RABBIT SERA

RABBIT NO.	STOCK ANTIGEN	T. PALLIDUM ANTIGEN	T. PERTENUE ANTIGEN	T. MICRODENTIUM ANTIGEN	CONTROL ANTIGEN	REMARKS
1	++	–	–	–	–	Active lesions
2	++	–	–	–	–	Active lesions
3	–	–	–	–	0	Recovered from syphilis
4	–	–	–	–	0	Recovered from syphilis
5	–	–	–	–	–	Recovered from syphilis
6	–	–	–	0	–	Recovered from yaws
7	–	–	–	–	–	Recovered from yaws
8	–	–	–	0	–	Normal rabbit
9	–	–	–	0	–	Normal rabbit
10	–	–	–	0	–	Normal rabbit
11	–	–	–	0	0	Normal rabbit

no reaction with the culture antigens occurred in any of the rabbit sera, although the two animals showing active lesions of syphilis gave absolute inhibition of hemolysis with the stock antigen. These results are in entire agreement with those of Noguchi, who found that his pallida antigen did not react with rabbit serum even when the animals presented active lesions of the infection. On the other hand, antigens prepared by extracting with alcohol the testicles of rabbits presenting a syphilitic orchitis will give positive results with syphilitic sera and also with the blood of rabbits injected with syphilis, thus proving that the alcoholic extract of cultures of *Treponema pallidum* is an entirely different antigen from that obtained from the infected testicle of rabbits and that the reactions obtained with such an extract are, at least, partly specific in character. In 1911, Nichols published some experiments of ours upon complement fixation with alcoholic antigens made from the testicles of rabbits suffering from experimental syphilis and yaws, and we found that while such antigens gave positive results with certain syphilitic sera, they also reacted with certain normal sera, as well as sera from other diseases, and that an antigen prepared from the testicles of rabbits inoculated with syphilis give positive results with the sera of rabbits inoculated with yaws

and *vice versa*. In addition, we found that alcoholic antigens prepared from the testicles of normal rabbits also gave positive results, so that we did not consider that this type of antigen was at all specific in character.

From our experiments with antigens prepared from pure cultures of the treponemas named we reached the following conclusions:

1. Alcoholic extracts of pure cultures of *Treponema pallidum* can not be depended upon in the diagnosis of syphilitic infection by the complement-fixation test, for many cases of undoubted syphilis, giving absolute inhibition of hemolysis with the stock antigen, an alcoholic extract of foetal syphilitic liver, gave a negative reaction with the culture antigen, and the reactions obtained with the pallidum antigen were generally weaker and less conclusive than with the stock antigen.

2. The results appear to prove that there are specific group reactions, in the way of complement fixation, or absorption, between *Treponema pallidum, Treponema pertenue* and *Treponema microdentium,* as antigens prepared from pure cultures of these organisms may all give complement fixation in the presence of syphilitic serum.

Later experiments[69] with antigens prepared from pure cultures of *Spirochæta refringens, Spirillum choleræ,* and *Bacillus typhosus* demonstrated that, while *Spirochæta refringens* sometimes reacted positively with the sera tested, the antigens prepared from the cultures of *Spirillum choleræ* and *Bacillus typhosus* never give a positive reaction, thus proving that the theory that positive reactions with these pure culture antigens might be due to lipoidal substances produced in the media itself by the growth of the organisms is practically untenable, and that there are group reactions among this class of organisms and that alcoholic extracts of these organisms contain some specific substance common to all, to a greater or lesser extent.

Since our work a considerable number of investigators have studied the immunological phenomena of cultivated

treponemas and spirochetes, and our results have been confirmed. Thus, Kolmer, Williams, and Laubaugh,[70] working with aqueous and alcoholic extracts of *Treponema pallidum* obtained positive reactions in secondary, tertiary, and congenital syphilis. They found that the aqueous extracts gave better results than the alcoholic but that, with both, the reactions were generally weaker than those obtained with the ordinary antigenic extracts used in the Wassermann test. They also obtained weak reactions with control antigens made from uninoculated media and other organisms. Zinsser, Hopkins, and McBurney[71] in an extended series of studies upon *Treponema pallidum* and syphilis obtained specific agglutination and complement fixation with culture treponemas but conclude that their work lends no support to the belief that either agglutination or complement fixation with cultures of *Treponema pallidum* will prove of any practical value in the diagnosis of syphilis. Finally, the work of Noguchi and Akatsu[72] proves that group reactions occur between certain treponemas and that specific immune bodies capable of producing agglutination, complement fixation, and opsonization could be produced in the blood serum of rabbits by injection of these organisms. They worked with *Treponema pallidum, Treponema calligyrum, Spirochæta refringens, Treponema microdentium,* and *Treponema mucosum,* and found that the *pallidum* produced higher degrees of agglutination and complement fixation, than the others, but that group reactions with complement fixation occurred between *Treponema pallidum* and *Treponema calligyrum,* between *calligyrum* and *Spirochæta refringens,* and between *Treponema microdentium* and *Treponema mucosum,* while there was slight group reaction between the latter and *Treponema pallidum.*

The present status of this subject is, that while specific complement fixation, at least within the limits of a group, is possible with either aqueous or alcoholic extracts of pure cultures of *Treponema pallidum,* and that aqueous extracts

give the best results there is no evidence sufficient to prove that such antigens are of real practical value in the diagnosis of syphilis by the Wassermann test. The reactions obtained with them are generally weak and doubtful and they are so often negative where the ordinary antigenic extracts are positive that their use in diagnosis is not justifiable. Further research may result in giving us a specific antigen for the Wassermann test but at present the outlook is very doubtful that such an antigen can be evolved.

CHAPTER VII

THE RESULTS OF THE WASSERMANN TEST IN THE VARIOUS STAGES OF SYPHILIS. TIME OF APPEARANCE OF THE REACTION. THE SPECIFICITY OF THE WASSERMANN TEST

The results of the Wassermann test vary with the different stages of syphilitic infection, the highest percentage of positive results being obtained in the secondary stage of the disease and the lowest in latent infections. The results also vary with the methods employed by different observers, although, in the hands of experienced serologists, the results obtained with one method are practically the same as those with another, and even in the hands of the inexperienced the results often are almost as good as when the test is done by the experienced investigator. It is one of the most fortunate things connected with this test, that it has given such accurate results with so many different modifications of the original test, and in the hands of such a great number of workers, many of whom were, and are, far from being experts in serology. As Vedder well says:[73] "One is impressed by the fact that the Wassermann reaction must be a test of most surprising merit to have survived all the clumsy technic that has been perpetrated in its name."

The figures as to the results of this test in the various stages of syphilis which are here given, aside from those given by authorities quoted, are based upon more than fifty thousand Wassermann tests personally made by the writer, using the method which has been recommended in Chapter V. A great many thousands of these tests were made upon conditions due to other causes than syphilis

while a still greater number were reexaminations made upon the same case, sometimes as many as twenty or more tests having been made upon a single patient, in the course of months or years. In the army it has been customary to control the treatment of syphilis by repeated Wassermann tests and this practice has not only enabled army surgeons to have scientific control of the treatment of their syphilitic patients but has also enabled us to have a very accurate check upon the efficiency and reliability of the modification of the Wassermann test which is recommended in this work. The writer has tried to keep himself informed as to its success in the hands of other laboratory workers and it is gratifying to be able to report that the modification has proved satisfactory in every laboratory in the army in which it has been adopted, and this includes nearly all, and that it has been recommended as a standard method for all army laboratories by Lieut. Col. Vedder,[74] who has devised special apparatus which makes such standardization possible.

Before considering the results of the Wassermann test in each stage of syphilis, it is well to call attention to the fact that many of the statistics which are to be found in the literature are based upon observations made during the first few years after the test was discovered and are, therefore, not representative of the results that are really being obtained today. A great amount of research has been expended upon the various reagents used in the Wassermann test and a great many factors have been discovered that influence the results of the test, in one way or another, so that at the present time much better results are being obtained with the test than formerly and many inaccuracies in technic have been corrected. This fact must be borne in mind in considering the percentages of positive results recorded by the earlier observers in the various stages of syphilis and especially the numerous reports of positive results in diseases other than syphilis. In fact, the results now being obtained with this test are hardly at

all comparable with those which were obtained with it during the first year or two after Wassermann's publication of his technic.

As an illustration of the results obtained with the author's method in the various stages of syphilis Table XXVI is inserted, giving the results of the test in 5,600 syphilitic infections, personally tested by him:

TABLE XXVI

THE RESULTS OF THE WASSERMANN TEST IN 5,600 CASES OF SYPHILIS

STAGE	TOTAL CASES	POSITIVE	%	NEGATIVE	%
Primary	1080	970	89.8	110	10.1
Secondary	2217	2132	96.1	85	4.9
Tertiary	728	633	87.4	95	13.5
Latent	1525	1039	68.1	486	31.8
Congenital	28	25	82.2	3	10.7
Parasyphilis	22	15	68.1	7	31.8
TOTALS	5600	4814	85.9	786	14

In the above cases no test was considered positive unless there was complete inhibition of hemolysis (a four-plus reaction) except in the very early primary cases when a three-plus reaction was considered positive in a few instances. By insisting upon absolution inhibition of hemolysis as indicating a positive reaction in these cases it is undoubtedly true that some cases of infection, which gave reactions almost absolute, have been erroneously considered as negative, but in most of the army laboratories we have not felt justified in basing a positive diagnosis of syphilis upon anything less than absolute inhibition of hemolysis. In considering this table it should also be remembered that in at least 95 per cent of the cases tabulated only one Wassermann test was performed and that had repeated tests been made it is undoubtedly true that a higher percentage of positive results would have been obtained in all of the stages of syphilis tested.

Our results are in practical agreement with those of most recent observers, so that it may be stated that about 86 per cent of infections with syphilis will give a positive

reaction with the Wassermann test, if they are distributed among the various stages as in the table given; that 10 per cent of primary cases will give a negative result; about 5 per cent of secondary cases; about 13 per cent in the tertiary stage; and about 30 per cent in the latent stage of the disease. With good technic and efficient antigenic extracts one should obtain 90 per cent of positive results in the primary stage of syphilis; 95 per cent of positive results in the secondary stage; 87 per cent of positive results in the tertiary stage; and 70 per cent of positive results in the latent stages.

Results in the Primary Stage of Syphilis.—It is now a generally accepted fact that the earlier a syphilitic infection is diagnosed the better will be the results of any method of treatment that may be instituted. Thus the early diagnosis of the disease becomes of paramount importance and with the dark-field apparatus and the Wassermann test we are supplied with agencies that, if properly used, should enable us to make the diagnosis during the early portion of the primary stage of the disease in the vast majority of infections.

If possible every patient exhibiting a sore upon the penis should be given the benefit of an examination of the serum from the sore by the dark-field apparatus. If this were done, the *Treponema pallidum* would be found in most instances of true syphilitic infection, and thus the disease would be diagnosed at a very early stage. However, the dark-field apparatus is often unobtainable and the examination can not be made, so that the question arises as to the diagnostic value of the Wassermann test in the primary stage of syphilis. If one were to believe some writers it would be concluded that during this stage of syphilis the Wassermann test is of very little value, as so many cases give a negative reaction, but this has not been the writer's experience with his modification of the test, for, as already mentioned, practically 90 per cent of the primary cases he has tested have given a positive result before the de-

velopment of secondary symptoms. Therefore, if a dark-field examination can not be made, or is negative, it is recommended that every patient presenting a suspicious lesion upon the penis be given a Wassermann test, for if modern research upon syphilis has proved anything, it is that the morphology of a sore upon the penis is of much less value than formerly supposed, and that the typical Hunterian chancre is almost as much the exception as the rule. Mixed infections of chancre and chancroid, with the resultant confusion in clinical pictures, have been quite common in the writer's experience, and with the admissions by the best authorities upon syphilis that it is often impossible to diagnose syphilis from the appearance of the initial lesion, the use of the Wassermann test and the dark-field apparatus in every suspicious case is imperative.

From experience and experimental observations it is certain that a syphilitic infection must exist for a certain length of time in man before the substance or substances which give rise to a positive Wassermann reaction appear in the blood. Just how long this is will vary with the virulence of the infection and the resisting power of the patient, but it is beyond controversy that sometimes the test proves positive a very few days after the appearance of the initial lesion, although this occurs but rarely. The writer has observed and reported[75] one case in which the reaction occurred only five days after the appearance of the chancre and two cases in which it occurred after eight days of the appearance of the initial lesion. As will appear in Table XXIX, no less than 27 of 77 cases, or 36 per cent of primary cases tested by the writer, gave a positive reaction during the first week after the chancre appeared and almost 60 per cent during the second week after the appearance of the chancre. It is thus evident, that even very early in the primary stage of syphilis a positive Wassermann reaction may occur, and thus the test will prove of value in diagnosis.

As would be expected, the statistics given by different au-

thorities regarding the percentage of positive results in the primary stage of syphilis vary greatly, especially those published shortly after Wassermann's original paper upon the subject. Kolmer[76] states that in general, in primary syphilis positive results will be found in from 80 to 90 per cent of the cases tested, and the writer agrees with this statement, as it has been his experience that when properly performed the Wassermann test should give at least this number of positive results. Table XXVII gives the results obtained by different observers in the primary stage of syphilis:

TABLE XXVII

RESULTS OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN THE PRIMARY STAGE OF THE DISEASE

NAME OF OBSERVER	NO. OF CASES	POSITIVE	% POSITIVE
Arning	48	25	60
Bering	56	47	84
Boas	50	30	60
Bruck-Stern	27	13	48.5
Fischer-Meier	8	6	75
Grosser	20	19	95
Hancken	17	15	88
Craig	1080	970	89.8
Kaplan	138	125	90
Noguchi	70	65	92.8
Swift	16	13	81
Vedder	242	178	73.5

From this table it is evident that the percentage of positive results with the Wassermann test in the primary stage of syphilis has varied greatly in the hands of different observers, the percentage being as low as 48 per cent and as high as 95 per cent. However, as already stated, from 80 to 90 per cent of the usual run of primary infections coming to the physician should give a positive reaction. Of course, if tests were made upon a very large number of very early infections in a series reported, the percentage would be much lower than if cases were tested from two to three weeks after the appearance of the initial lesion, but the writer's experience has been that at least 80 per cent of cases of primary syphilis should give a positive

Wassermann test and it is believed that, in the army, the initial lesion is reported to the surgeon at least as soon as it is, in the vast majority of cases, in civil life.

It is true that the percentage of positive results with this test varies more in the primary stage of syphilis than in other stages, with the possible exception of the latent stage, due to many reasons, among the most important being that the test is made within a very few days of the appearance of the initial lesion or early treatment often obscures the reaction at this stage of the disease. The writer has often observed negative cases become positive with the cessation of treatment, during the primary stage, and hundreds of instances in which, in untreated cases, a negative reaction became positive after an interval of days or weeks, and before the occurrence of secondary symptoms. It should be remembered, however, that a certain proportion of negative results must be expected in the primary stage of syphilis and that a negative result is no proof that a suspicious lesion is not syphilitic.

Time of Appearance of the Reaction

The date after infection with syphilis that one may expect the Wassermann reaction to be positive is a point of great practical interest, for the earlier treatment is commenced in syphilis the better are the therapeutic results, and unless the Wassermann test can assist in diagnosis early in the disease, it loses much of its value as a diagnostic agent. While the ideal method of diagnosing syphilis in the primary stage is by the demonstration of *Treponema pallidum* with the dark-field apparatus, this measure is not infrequently unsuccessful, especially if local treatment has been given the initial lesion, and, in addition, comparatively few practitioners possess the apparatus. The demonstration of the treponema in stained preparations or by the India ink method should always be attempted but these methods are also often unsuccessful, so

that one must depend upon the Wassermann test frequently for an early diagnosis.

Contrary to the opinion of some observers, the writer believes that this test is a most valuable aid to the diagnosis of syphilis even thus early in the disease, for, in his experience, nearly 90 per cent of primary cases tested by him have shown a positive result before the appearance of secondary symptoms, and while a single test performed at a very early date after the appearance of the chancre will be negative in a very large proportion of cases, repeated tests will prove that the reaction becomes positive before the appearance of secondary symptoms in most patients.

In Table XXVIII Vedder[77] shows the results of the examination of cases of syphilis in the primary stage by both the dark-field apparatus and the Wassermann test, and it is of interest as demonstrating that both methods fail, at times, to aid in the diagnosis of the disease.

TABLE XXVIII

RESULT OF EXAMINATION OF PRIMARY CASES OF SYPHILIS BY DARK-FIELD AND THE WASSERMANN TEST*

DURATION OF SORE	DARK-FIELD	WASSER-MANN	DURATION OF SORE	DARK-FIELD	WASSER-MANN
2 days	+	–	1 month	–	++
3 days	+	–	7 days	+	++
7 days	+	–	9 days	+	+
7 days	+	–	14 days	+	+
10 days	+	–	15 days	+	+
2 weeks	+	–	1 month	+	++
2 weeks	+	–	1 month	+	+
3 weeks	+	+–	1 month	+	++
3 weeks	+	–	1 month	+	++
3 weeks	+	–	1 month	+	++
1 month	+	–	1 month	+	++
1 week	–	++	1½ months	+	++
1 week	–	+	2 months	+	++
2 weeks	–	++	1 month	–	–
3 weeks	–	+	1 month	–	–
1 month	–	++			

*The sign ++ equals absolute inhibition of hemolysis, the four-plus reaction of many laboratories.

Table XXVIII is conclusive evidence of the value of the Wassermann test as an aid in the diagnosis of syphilis in

the primary stage of syphilis. It will be noted that in no less than three cases the Wassermann test was positive one week after the appearance of the chancre and that in two of these cases the dark-field examination was negative. The table also demonstrates that in every case of primary syphilis both a dark-field examination and a Wassermann test should be made, if possible.

Table XXIX, compiled from personal tests, gives the date of appearance of the positive reaction in periods of weeks after the appearance of the initial lesion. Six hundred cases of primary syphilis are here considered and the Wassermann tests were performed by the writer in the ordinary course of routine examinations in the army. A great many more cases of primary infection were really tested but are not included for the reason that we could not be sure of the data regarding the date of appearance of the initial lesion.

TABLE XXIX

DATE OF APPEARANCE OF WASSERMANN REACTION IN WEEKS IN 600 CASES OF PRIMARY SYPHILIS

WEEK AFTER APPEARANCE OF CHANCRE	TOTAL CASES	POSITIVE	%	NEGATIVE	%
First week	77	27	36.3	50	64.9
Second week	155	92	59.3	63	40.3
Third week	158	109	68.9	49	31.
Fourth week	167	129	77.2	38	22.7
Fifth week	43	35	81.3	8	18.6

From this table it is evident that, in the writer's experience, 36 per cent of cases of primary syphilis have given a positive Wassermann reaction by the end of the first week after the appearance of the initial lesion; almost 60 per cent by the end of the second week; almost 70 per cent by the end of the third week; over 77 per cent by the end of the fourth week; and over 80 per cent by the end of the fifth week after the appearance of the chancre.

It is also evident that this test is of very distinct value in the diagnosis of syphilis during the primary stage of

the disease, and should always be resorted to if a dark-field examination is impossible or gives a negative result. A single negative test, at this stage especially, should not be considered as proving that a suspected lesion is non-syphilitic, but repeated tests should be made before a patient is assured that the condition present is not due to syphilis. In the army, owing to the situation of the various Department Laboratories, the Wassermann test is always available and suspected cases can be examined at weekly intervals, a practice which enables the army surgeon to establish the diagnosis very early in the disease, but in civil practice this is generally impossible, owing to the cost of the test. However, whenever possible, repeated Wassermann tests should be made upon patients suspected of presenting the primary lesion of syphilis.

As regards the question as to how early the positive Wassermann may appear in the blood after the primary lesion appears, there are a considerable number of observations upon record showing that positive reactions have been obtained as early as the third and fourth days after the appearance of the chancre, and a few in which a positive reaction has been reported even before the appearance of the initial lesion. In view of our knowledge of the nature of the syphilitic poison it may be stated that one must regard with suspicion the reports of a positive Wassermann before a primary lesion was observed, for it is practically impossible to rule out previous syphilitic disease in most patients and unless this can be done or it can be definitely proved that the lesion supposed to be primary in such cases is really so, by the demonstration of *Treponema pallidum,* the evidence can not be considered as conclusive.

However, it may be accepted as proved beyond a doubt that the Wassermann test is not so very infrequently positive by the end of the first week after the appearance of the chancre, and the writer has observed two cases in which it was positive five days after the sore was first noticed,

and in both cases *Treponema pallidum* was demonstrated in serum from the lesions. Well authenticated cases are on record in which the test became positive two or three days after the initial lesion was noted, so that the possibility of a positive Wassermann test before the appearance of such a lesion can not be denied, although it must be exceedingly rare.

It is now generally conceded that when the primary lesion of syphilis appears the disease has already become a generalized infection, so that it is not difficult to understand why a positive Wassermann reaction may occur very soon after the chancre appears, and the variation that occurs as to the time of the appearance of the positive reaction in different patients must depend upon the virulence of the treponema and the reaction of the tissues of the patient to the organism. The writer has observed many cases in which repeated Wassermann tests always proved negative, though there was no question of the presence of syphilis and marked lesions were present at the time that the tests were made, so that in a certain percentage of cases of syphilis it is probable that a positive reaction will never be obtained. The writer has also observed two patients in whom the primary lesion of syphilis was present, as demonstrated by the finding of *Treponema pallidum* by the dark-field, and whose blood serum gave a positive reaction prior to the development of secondary symptoms, but in whom no secondary symptoms ever developed, although they were under observation for nearly one year, and in whom the Wassermann reaction became negative, although no specific treatment was ever administered. In his opinion, these cases demonstrate that syphilis is sometimes spontaneously cured, and it is not improbable that this occurs much more frequently than is generally supposed.

In view of the reports of positive Wassermann reactions occurring before the initial lesion appears, the recent work of Woods and Morris[78] upon complement fixation in trypanosomiasis is very suggestive. They found that with

an antigen prepared from the spleen of a rat heavily infected with *Trypanosoma equiperdum*, complement fixation usually appeared in an infected animal's blood serum within eight days after inoculation, usually at the time that the trypanosomes appeared in the blood of the infected animal, but sometimes even before this occurred. In every instance, however, complement fixation preceded the appearance of symptoms of the infection.

Results in the Secondary Stage of Syphilis.—It is in the secondary stage of syphilis that the highest percentage of positive results are obtained with the Wassermann reaction. The per cent of positive reactions reported by various observers varies somewhat, but it may be stated that in this stage of the disease practically 95 per cent of the patients should give a positive result, provided, of course, that treatment has not been administered, but even in such cases one would expect from 90 to 95 per cent of positive results. Kolmer[79] states that in untreated secondary syphilis he has never obtained a negative result and Boas[80] in 437 untreated secondary cases obtained 100 per cent of positive reactions. The writer, in 2,217 cases of secondary syphilis, many of whom had received some specific treatment, obtained 2,132 positive results, or 96.1 per cent, and Vedder,[81] in 310 secondary cases of similar nature, obtained 91 per cent of positive results.

Table XXX gives the results obtained in the secondary stage of the disease by various observers:

From this table it will be noted that the percentage of positive results in the secondary stage of syphilis varies, according to different observers, from 79 per cent to 100 per cent, but it should be remembered that many of the cases tested by one observer may have had specific treatment, while those tested by another had had no treatment whatever, so that the low percentages reported can not be considered as giving any indication of the real value of the test in the absence of data regarding specific treatment.

TABLE XXX

THE RESULTS OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN THE SECONDARY STAGE OF THE DISEASE

OBSERVER	NO. OF CASES	POSITIVE	% POSITIVE
Arning	107	99	93
Bering	113	111	98
Bruck-Stern	115	101	87.1
Blumenthal-Roscher	131	130	99
Craig	2217	2132	96.1
Hoene	376	260	79.1
Lesser	204	186	91
Lederman	110	108	98
Merz	377	366	97
Noguchi	197	190	96
Schonnefeld	112	112	100
Swift	76	70	92
Vedder	310	285	91.9

As already stated, the percentage of positive results in untreated cases of secondary syphilis should be something over 95 per cent, and unless as high a percentage as this is obtained the complement-fixation system used should be carefully studied for the occurrence of possible errors, as the Wassermann test, as at present performed, should not be given a lower percentage of positive results in the secondary stage of syphilis than that mentioned.

A negative reaction in this stage of syphilis, when there is a clear history of infection and lesions are present, is of no value whatever in excluding the disease; and as negative reactions may occur, even when the test is repeated many times during this stage of the disease, the diagnosis of syphilis should be made, in such instances, without reference to the result of the test. It should also be remembered that the severity of the lesions present has nothing to do with the type of reaction obtained, for cases with very mild symptoms will frequently react as strongly as those showing severe lesions, and not infrequently a patient presenting the most pronounced secondary eruption and mucous patches will give a weak Wassermann reaction, which, unless repeated, might be interpreted as doubtful. However, in these cases repeated Wassermann tests almost

invariably result in the appearance of a strong positive reaction.

Results in the Tertiary Stage of the Disease.—Perhaps in no other stage of syphilis has the Wassermann test proved as valuable as in the tertiary stage, and especially in those cases in which there is involvement of the central nervous system. The percentage of positive results obtained will vary, of course, with the amount of treatment that has been given the patient, for in a large majority of the cases of tertiary syphilis, observed in practice, a considerable amount of specific treatment has been administered prior to the time that the blood was collected for a Wassermann test. If cases are tested that have received no specific treatment and in which active tertiary lesions are present, the percentage of positive results should be above 95 per cent, but in the usual run of cases examined the Wassermann test, if properly applied, should give positive results in from 80 to 85 per cent of cases examined. The writer, in 728 cases of tertiary syphilis obtained 633 positive results, or 87.4 per cent, using the method recommended in this work, and Vedder,[82] using the same method, obtained 227 positive reactions in 263 cases tested, or 86.3 per cent.

Table XXXI gives the results reported by various ob-

TABLE XXXI

RESULTS OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN THE TERTIARY STAGE OF THE DISEASE

OBSERVER	NO. OF CASES TESTED	POSITIVE	% POSITIVE
Arning	30	27	90
Bering	45	37	82
Bruck-Stern	47	27	57
Fleishman	41	40	98
Hohne	33	21	63
Craig	728	633	87.4
Lederman	78	75	96
Lesser	131	119	90
Merz	158	127	80.3
Noguchi	177	159	89.9
Swift	45	37	80
Vedder	263	227	86.3

servers of the complement-fixation test for syphilis during the tertiary stage of the disease.

From this table it is evident that the results of the Wassermann test in the tertiary stage of syphilis varied from 57 per cent to 96 per cent, according to various observers. This great variation in the results may probably be explained by certain of the observers including under the tertiary stage cases tested, 1, cases without definite symptoms; 2, cases that had received specific treatment within a recent date or were receiving treatment; and 3, cases in which tertiary symptoms had never appeared. It is evident that, until statistics are based upon similar cases a great deal of variation must be expected in the results of this test in the hands of different observers.

In syphilitic disease of the blood vessels, as aortitis, aortic aneurism, and aortic insufficiency, the Wassermann test has proved of the very greatest diagnostic value and in syphilitic disease of the central nervous system the test is invaluable. This subject will be discussed more fully in Chapter X.

Results in the Latent Stages of the Disease.—The Wassermann test in the latent stages of syphilis has been of the greatest use in indicating that treatment was necessary in patients thought to be cured of the infection because of the absence of symptoms for long periods of time. There is probably no disease occurring commonly in the practice of the physician which so often exhibits intervals during which clinical symptoms are absent as does syphilitic infection and often a positive Wassermann reaction is the only tangible evidence of the disease. The pathological investigations of careful workers, as Warthin, have shown that the human body may be extensively invaded by *Treponema pallidum* without clinical symptoms being evident and with a positive Wassermann reaction as the only evidence of such infection, and one of the most valuable of assets of the Wassermann test is its capacity for detecting such infections. Of course, the test is frequently negative

in this class of patients, as would be expected, for, as the writer has shown, a certain amount of the substance or substances causing complement fixation in syphilis must be present in order to accomplish this result, and in the latent cases of syphilis a sufficient amount is not present in a considerable percentage of cases, as shown by the results of the test.

The writer has tested 1,525 cases of latent syphilis, with a positive reaction in 1,039, or 68 per cent, using the method of performing the test recommended in this work. Vedder,[83] using the same method, tested 114 cases with a positive result in 92, or 80.7 per cent. Boas,[84] in 363 cases of early latent syphilis obtained positive results in only 40 per cent of the cases, while in older cases he obtained positive results in but 22 per cent of treated cases but in cases treated carelessly the positive results equaled 74 per cent.

Table XXXII gives the results of the Wassermann test obtained by the observers mentioned in the latent stage of syphilitic infection.

TABLE XXXII

RESULT OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN THE LATENT STAGES OF THE DISEASE

OBSERVER	NO. OF CASES TESTED	POSITIVE	% POSITIVE
Bering	147	75	48
Grosser	35	12	33.3
Craig	1525	1039	68.1
Fox	54	25	46
Lederman	78	36	46
Noguchi	265	206	77.7
Swift	39	25	64
Vedder	114	92	80.7

As would be expected, there is a great variation in the number of positive reactions obtained with the test in the latent stages of syphilis, as shown in this table. When it is remembered that in most of the cases tested specific treatment had been given for a longer or shorter period of time; that there were no symptoms of the disease present at the time the blood was tested; and that in some the

only history of a syphilitic infection was a doubtful one; the low percentage of positive results sometimes obtained in this class of cases can be understood. In the writer's cases there was always a clear history of infection but in the majority of the patients some specific treatment had been taken, so that the percentage of positive results obtained; i. e., 68 per cent, may be considered the average percentage obtained in latent infections in such cases.

The value of the Wassermann test in the diagnosis of syphilis in the latent periods of the disease can not be overestimated either from the sociological or therapeutic standpoint. The sociological aspect of latent syphilitic infection will be treated of more fully in the discussion of the subject of Wassermann surveys, but it can not be too strongly emphasized that there is a very large amount of syphilitic infection present in all countries that is never recognized and it has only been within very recent years that, because of researches upon various classes of the population by means of the Wassermann test, it has been realized to what a great extent this insidious disease has invaded society.

From the therapeutic standpoint, the Wassermann test is most valuable in the latent stage of syphilitic infection because a positive reaction is the only index which we possess as to the existence of the infection and the necessity for specific treatment. The test is often positive even after specific treatment has been administered for months and years, and the vast majority of patients treated with mercury, before the days of salvarsan, and supposed to have been cured, will give a positive reaction with the Wassermann test. Some authorities have gone so far as to state that they do not believe that any syphilitic infection was ever cured by mercury alone, because of the revelations of the Wassermann test in latent infections, but the writer believes this to be too strong a statement, although it must be admitted that the cases proving the contrary are comparatively few in number.

Results in Congenital Syphilis.—The results of the Wassermann test in congenital syphilis vary with the character of the case tested. In children showing the lesions of syphilis at birth the test gives 100 per cent of positive results, in the writer's experience, but in children exhibiting the lesions of late congenital syphilis the percentage of positive results is lower, averaging from 80 to 85 per cent. In still older individuals in whom the disease is supposed to be congenital the percentage of positive results will not exceed 70 to 75 per cent.

Table XXXIII gives the results obtained by several observers with the Wassermann test in congenital syphilis:

TABLE XXXIII

RESULTS OF THE COMPLEMENT-FIXATION TEST FOR SYPHILIS IN CONGENITAL SYPHILIS

OBSERVER	NO. OF CASES TESTED	POSITIVE	% POSITIVE
Arning	5	3	60
Craig	28	25	82.2
Jesionek-Meirosky	18	16	88.8
Noguchi	17	17	100
Vedder	128	123	96

The variation obtained by different observers in the results of the Wassermann test in congenital syphilis are well shown in the table and depend upon the causes already mentioned. The recent work of Veeder[85] upon the subject is of interest. He studied 100 syphilitic families with the following results: In the 100 families, 331 pregnancies occurred resulting as follows: abortions, 100, or 30.2 per cent; stillbirths, 31, or 9.3 per cent; living births, 200, or 60.5 per cent. Of the 200 living births, 39 of the children had died at the time of the investigation and of the 161 living children, 12 died during the course of the investigation. Of the 161 tested, 107 had both clinical symptoms of syphilis and positive Wassermann reactions; 5 were clinically positive but gave negative Wassermann tests; 16 were clinically negative but gave positive reactions, leaving 33 children who were both clinically negative and

who gave a negative Wassermann test. In this series of cases of congenital syphilis in young children born of syphilitic parents Veeder obtained 96 per cent of positive results, and his work well illustrates the value of the test in the diagnosis of congenital infection.

The Wassermann test has changed our conception of Colles' and Profeta's laws and has explained these apparent contradictions in the etiology of syphilis. Colles' law, that an apparently healthy mother of a syphilitic child may suckle the child with impunity, even though it presents the most infectious lesions of the disease, is explained by the fact that the mother, although showing none of the lesions of syphilis is really in the latent phase of the disease, as demonstrated by the positive Wassermann reaction that is almost invariably obtained with the blood of such mothers. Profeta's law, that the child born of a syphilitic mother, but presenting no evidence of the disease, may suckle its mother with impunity, has also been shown by the Wassermann test to be due to latent syphilis in the child, as in these children the test is generally positive although no symptoms of syphilis are present and none may appear for long periods of time. In other words, children born of syphilitic parents possess no immunity to syphilis as was long believed, nor does a mother who with impunity suckles her syphilitic child, possess an immunity, but is herself syphilitic.

Just what part late hereditary syphilis plays in the numerous instances in which a positive Wassermann reaction is found in patients who deny any syphilitic infection and who may, or may not, present evidences of the disease, is still a mooted question. In the writer's experience it has not infrequently occurred that patients who are absolutely truthful and who have given a positive Wassermann reaction, have denied even any exposure to infection or any lesions that could be considered syphilitic, and there has been no reason to doubt the truth of their statements. In

such cases, especially if there are no lesions of the disease present, one should be very careful to eliminate any condition that has been proved to give a positive Wassermann reaction, but after this has been done the explanation of the reaction is often very difficult and even impossible. That hereditary syphilis, latent in character, is the cause of some of these reactions can not be doubted but the writer is loth to believe that most of them are due to such a cause. It is more probable that an accidental infection may have occurred in infancy or childhood, the initial lesion of which went unrecognized, than that the positive test is due to a hereditary infection that has never caused symptoms or lesions of syphilis.

The Specificity of the Wassermann Reaction

While it must be admitted that the Wassermann reaction is not absolutely specific for syphilis, for positive results have sometimes been observed with other diseases, the fact remains that a positive reaction with this test indicates syphilis in so immense a proportion of individuals giving it that the practical value of the reaction in the diagnosis of syphilis is hardly at all decreased by the comparatively few instances in which such a result is obtained in other conditions.

If one reads the works of writers published shortly after the discovery of the Wassermann test, the attention is at once called to the large number of conditions other than syphilis in which positive reactions are reported; but if these writings be compared with those published by investigators during the past five years, it is evident that most of the so-called positive reactions must have been due to poor technic, for as our knowledge of the technic has increased, these nonspecific reactions have decreased in number, until today a positive reaction with the test is reported in but few conditions that are not due to syphilis. Any serologist who reports any considerable number of

positive reactions with the Wassermann test in diseases other than syphilis proclaims that his technic is poor, for, if properly performed, this test will give positive results in very few nonsyphilitic conditions.

The earlier writers upon the subject reported positive results in such diseases as scarlet fever, septicemia, diphtheria, carcinoma, and most of the acute infections; but it is now well known that these conditions do not give a positive reaction if the test is properly performed. There is still an opinion prevalent among the profession that the test is frequently positive in scarlet fever, but the work of recent investigators proves conclusively that the test is not positive in this disease but that when a positive reaction does occur the child is syphilitic or syphilis can not be excluded. Hecht, Lateiner, and Wilenko[86] tested 105 cases of scarlet fever and obtained but one positive result, and Kolmer[87] in 250 cases which he tested with both the original Wassermann method and the Noguchi modification obtained positive reactions in only 5 cases, or 2 per cent, and in these cases syphilis could not be excluded. Browning and McKenzie[88] examined 37 cases of scarlet fever and did not obtain a positive reaction in one of them. These results prove that the Wassermann test is not positive in scarlet fever and that the large proportion of positive results reported in the early literature of the test must have been due to improper technic.

Another disease in which a considerable proportion of positive reactions have been reported is tuberculosis. Some authorities have reported as high as 30 to 40 per cent of positive results in this disease but such reports are absolutely unreliable and prove that the method of performing the test must have been erroneous. The writer has tested hundreds of cases of tuberculosis and while a few have shown a positive reaction, syphilis could not be excluded in any case, and the majority of the patients admitted infection. There are many instances on record of syphilis of the lung existing along with tuberculosis of

the same organ and many in which syphilis of the lung was diagnosed as tuberculosis, and it is these cases that have caused the impression that tuberculosis frequently gives a positive reaction with this test, together with the large number of latent syphilitic infections which are found in patients suffering from other diseases. There is also considerable evidence to prove that tuberculosis renders an individual much less resistant to infection with *Treponema pallidum* and this probably has much to do with the prevalence of the disease in the tubercular. However, that tuberculosis is a disease which causes a positive Wassermann reaction can not be longer maintained in view of the negative results of the best observers as reported in the most recent literature.

There are, however, a few diseases in which a positive Wassermann reaction is sometimes obtained and where the most careful examination fails to disclose any evidences of syphilitic infection and the most careful inquiries any history of the disease. These diseases are yaws or frambesia; the tubercular type of leprosy; some cases of relapsing fever; some malarial infections, during the febrile stage; and some instances of experimental trypanosomiasis in animals. Of these, yaws is the most important because some of the lesions of this disease resemble those of syphilis and in the lesions the causative organism, *Treponema pertenue,* may be found either by a dark-field examination of the secretions from the lesion or by staining methods. As this treponema is almost indistinguishable morphologically from *Treponema pallidum,* and the blood often gives a positive Wassermann reaction, some confusion in diagnosis may result, but a careful study of the case should enable the diagnosis of yaws to be made from the clinical history and symptoms alone.

The writer has observed positive reactions in five cases of tertian malarial infection, during the febrile stage of the paroxysm, the blood becoming negative during the

afebrile periods. Thompson[89] states that he has observed positive results several times in malarial disease during the stage of fever which became negative with the disappearance of the fever and plasmodia, and our results have been confirmed by many observers. On the other hand, the occurrence of a positive reaction in malaria, even during the paroxysm of fever, is comparatively rare, for the writer has examined many cases in which the test was negative and other investigators report similar results. However, the microscopical examination of the blood should suffice to diagnose malarial infections, so that the occurrence of a positive Wassermann reaction should cause no trouble in the diagnosis of the disease. If the reaction persists after the disappearance of the fever and plasmodia the diagnosis of syphilis should be made, for all investigators report that the positive reaction in malaria is only temporary in character.

In the tubercular forms of leprosy numerous positive reactions have been reported but it is impossible to say just how many of these were true false reactions and how many were due to a complicating syphilitic infection. If it be remembered that most of the cases of leprosy that have been tested occurred in native races which were thoroughly syphilized the difficulty of ruling out that infection can be appreciated. The writer believes that a positive reaction does occur in tubercular leprosy in certain stages of the disease but he also believes that the majority of the positive reactions reported in leprosy have been due to a concurrent syphilitic infection. In the anesthetic form of leprosy the Wassermann test is generally negative, so far as reports indicate. As tubercular leprosy can be easily diagnosed by the finding of the bacillus in the lesions a positive Wassermann reaction should occasion no confusion in the diagnosis.

Cases of relapsing fever have been found to give a positive reaction and also some experimental infections with trypanosomiasis but it is yet unsettled whether or not the

blood of patients suffering from infection with *Trypanosoma gambiensi* or other human trypanosomes gives a positive reaction.

Positive reactions have been reported in diabetes mellitus, in cases suffering from acidosis, and in pellagra, but so far as the latter disease is concerned it is now definitely proved that the Wassermann test gives only negative results.

As regards the number of positive reactions occurring in diseases other than syphilis it may be stated that, with a properly performed and controlled method of making the test, it will be so small as to be of practically no importance from a diagnostic standpoint. The writer has obtained 12 positive reactions among 4,000 individuals suffering from diseases other than syphilis or 0.3 of 1 per cent. Of these patients five were suffering from tertian malarial infection and gave a positive result during the febrile stage, which became negative when the temperature returned to normal; three were diagnosed as tuberculosis; three as pityriasis rosea; and in one the diagnosis was undetermined. In the pityriasis rosea cases the reactions were almost double-plus (four-plus), but would not have been reported as positive without a qualifying statement, while in two of the tuberculosis cases a history of syphilitic infection was afterwards obtained, and the disease could not be excluded in the remaining case. But, even admitting that all of these cases gave a false positive reaction, the real value of the test is but very little affected as a diagnostic measure. Vedder[90] has obtained results almost identical with those of the writer, using the writer's modification of the Wassermann test, as he obtained only four positive reactions among 1,049 individuals suffering from diseases other than syphilis.

When it is remembered how difficult it is to absolutely exclude a syphilitic infection in any individual and the comparatively few instances in which the Wassermann test gives a positive result in diseases other than syphilis, one

should accept with great caution the reports of positive reactions in such diseases, for the experience of those who have worked with this test for years and in hundreds and thousands of cases has been that a positive reaction, in the overwhelming majority of instances, demonstrates the presence of syphilis. There are a very few conditions in which a positive reaction is obtained with this test and in which syphilis can be eliminated, but as most of them can be easily diagnosed by laboratory and clinical methods, there should be no confusion caused by the result of the Wassermann test, but even in these cases a very careful inquiry and examination should be made before a possible complication of syphilis is excluded.

In surgical practice weak positive reactions are sometimes noted in blood sera collected shortly after ether and chloroform anesthesia, so that the test should not be made upon patients who have been anesthetized until at least two days have elapsed since the anesthetic was administered.

In conclusion, it may be stated that the substance or substances that cause a positive Wassermann reaction appear to be practically peculiar to the blood serum of patients suffering from syphilitic infection, and while it can not be claimed that the test, when positive, is absolutely specific of syphilis, from a practical standpoint it is doubtful if a more specific test is employed in medicine, the margin of error appearing to be less than five-tenths of one per cent.

CHAPTER VIII

THE INTERPRETATION OF THE RESULTS OF THE WASSERMANN TEST. THE WASSERMANN TEST AS AN INDEX OF THE PREVALENCE OF SYPHILIS IN COMMUNITIES

The writer has often been impressed with the evident misconceptions of many practitioners of medicine regarding the nature of the Wassermann reaction, the percentage of positive results that may be expected in the various stages of the disease, but, especially the exact significance that may be attached to a positive or negative result. In his experience, many patients have been told by their medical attendant that they were free from syphilis, the statement being based upon a single negative result with this test, while others have been told that they were suffering from the disease upon the strength of a plus or plus-minus reaction, in the absence of either a history of infection or of any symptoms of the disease.

Such interpretations of the results of the Wassermann test are unwarranted and have brought the test into disrepute in certain localities. In addition, innocent individuals have suffered great mental anguish from being unjustly stigmatized as afflicted with syphilis, and others have been infected by those who have been told that they were free from the disease by some careless or ignorant practitioner. Even today, almost ten years since the Wassermann test has been in general use by the profession, there is a surprising amount of ignorance regarding the interpretation of the various grades of the reaction, as reported by different laboratories, and much of the fault lies with the laboratory reports, for the terms strongly positive, positive, and weakly positive, so commonly used,

are misleading, unless they are carefully explained in reference to each case. In fact, the writer has always believed that only three terms should be used in reporting the results of the Wassermann test; i. e., *positive, doubtful,* and *negative,* leaving it to the clinician to decide how much weight should be attached to any grade of the reaction which is reported as doubtful, and which, in the army, would mean any degree of the reaction less than complete inhibition of hemolysis. While there is no doubt that many cases of syphilis react weakly consistently, especially in the early and late latent stages, never presenting complete inhibition of hemolysis, the writer believes that all cases showing less than complete inhibition should be reported as doubtful, and the interpretation of the reaction left with the clinician. Of course, if there is a clear history of infection and typical clinical signs are present, the doubtful reaction would at once, in the opinion of the clinician, be considered as positive and as supporting the diagnosis of syphilis, but if, on the other hand, there was no history of infection and no symptoms are present, a doubtful Wassermann reaction is not sufficient proof upon which to base a diagnosis of syphilitic disease.

The statements here made regarding the interpretation of the results of the Wassermann test are based upon over 50,000 tests personally performed by the writer, the majority of them being reexaminations made as a control of the treatment of the disease, the remainder being upon patients presenting the symptoms common to the various stages of syphilis. The percentage of positive and negative results obtained in these cases has already been given (Chapter VII), as well as the terminology used in reporting the results, but it is well here to repeat the terminology used in the army laboratories.

Four degrees of the reaction are noted in reports from the army laboratories. A positive reaction is reported as double-plus (++) and means that there was absolute inhibition of hemolysis. A doubtful reaction is reported as

plus (+) or plus-minus (+−), the former term indicating that there was over 50 per cent inhibition of hemolysis, the latter that there was less than 50 per cent inhibition of hemolysis. A negative reaction is reported as minus (−). In most civilian laboratories the results of the Wassermann test are reported as four-plus (++++), three-plus (+++), two-plus (++), plus (+), plus-minus (+−), and negative (−). The *four-plus* reaction corresponds to the army *double-plus;* the *three-plus* and *two-plus* to the army *plus;* and the *plus* and *plus-minus* to the army *plus-minus.*

These distinctions between the terminology used should be borne in mind in the following discussion of the interpretation of the results of the test.

The writer has already discussed the specificity of the Wassermann reaction (Chapter VII) and there remains the question as to the significance of the *positive* reaction. Some authors have denied that a positive reaction with this test indicates the presence of syphilis, claiming that after the disease disappears the reaction still persists, and that the positive result is simply an indication that the patient has had syphilis sometime in the past. In the light of modern research upon the disease this theory can no longer be considered as worthy of credence, for it is the unanimous experience of those who have had the most to do with the treatment of syphilis, as controlled by the Wassermann test, that with the disappearance of the treponema and of symptoms the reaction also disappears and that, in cases that are really cured, the reaction remains permanently absent. That this is true has been proved experimentally upon infected animals, and the disappearance of the reaction in these animals after the administration of salvarsan which has caused the disappearance of the treponemas, is conclusive evidence that the positive reaction does not persist after the cure of the syphilitic infection.

There are still others who maintain that, in the absence of symptoms, a positive Wassermann reaction does not indicate syphilis. To those who still hold to this opinion, the

writer has only to say that all experience has shown that, if the few conditions in which a positive result sometimes occurs can be eliminated, *a positive reaction means the presence somewhere in the body of living treponemas* and that the question of history or symptoms should have no influence in deciding the advisability of treatment. It is just this class of cases that later furnish the great bulk of our patients suffering from paresis, tabes, aortitis, aneurism, and syphilitic disease of the viscera, the latter often only discovered at autopsy. In cases in which there is good reason to question the possibility of syphilis the patient's blood serum should be titrated, for, as Vedder[91] well says:

"One-tenth of a cubic centimeter of some sera is required to give a double-plus reaction, while one-thousandth of a cubic centimeter of another serum may be sufficient to give a double-plus reaction. There would be little hesitation in making a diagnosis of syphilis in the latter case whether clinical symptoms were present or not. There might be doubt in the former case."

As the interpretation of the results of the test varies in the different stages of the disease, each stage will be considered separately.

The Interpretation of the Results of the Wassermann Test in the Primary Stage of Syphilis.—It is in this stage of the disease that the weaker grades of reaction are of most value from a diagnostic standpoint. As would be expected, the earlier in the primary stage the blood is tested, the larger will be the percentage of negative results and also the larger the percentage of doubtful reactions. As a certain amount of complement-binding substance must be present to give a positive result, the longer the infection has lasted the greater the chance that this amount is present and that the test will be positive. This fact has already been shown in the table giving the date of the appearance of the positive Wassermann reaction in 600 cases of syphilis, but, with a clear history of infection and a

suspicious lesion, it is proper to consider as positive, in this stage of the disease, reactions that would be always doubtful at other stages.

A *plus-minus* reaction (plus of most laboratories) should be considered as a negative reaction, in most instances, but if such a reaction is obtained within a week or two after the appearance of the initial lesion it may be considered as of some confirmatory value, but never as diagnostic of syphilis.

A *plus* reaction (+), the double-plus (++), and three-plus (+++) reaction of other laboratories, when obtained in the primary stage of syphilis, may be interpreted in practice as a positive reaction, provided there is a history of exposure and a suspicious lesion is present. The writer often obtains this type of reaction in cases in which the initial lesion has lasted for from one to three or four weeks, and he has never seen a plus reaction in such cases become negative, except as the result of treatment, and if untreated, the patients invariably developed secondary symptoms. In the absence of a history of infection or the initial lesion a plus reaction should not be considered as diagnostic of syphilis.

A *double-plus* (++) reaction, the four-plus (++++) reaction of some laboratories, is always diagnostic of syphilis in this, as in every other stage of the disease, provided the other conditions that sometimes give such a reaction, can be eliminated. This type of reaction, which means absolute inhibition of hemolysis, in the writer's experience has occurred in 14 per cent of the cases of primary syphilis tested during the first week after the appearance of the initial lesion; in 22 per cent tested during the second week; in 41 per cent tested during the third week; in 53 per cent tested during the fourth week; and in 61 per cent of cases tested during the fifth week after the appearance of the initial lesion. Reactions so strong as to almost equal complete inhibition of hemolysis occur in a large number of

cases of primary syphilis and these should be reported as double-plus or four-plus reactions. In these, the supernatant salt solution, after the undissolved corpuscles have settled to the bottom of the test tube, shows a slight reddish yellow tinge, demonstrating that a small amount of hemoglobin has been dissolved from the erythrocytes, but practically such reactions are positive and should be reported as such.

A *negative* reaction, in the primary stage of syphilis, is of no value whatever in eliminating syphilis. Even though it persists until the initial lesion disappears and during the latent stage before the occurrence of secondary symptoms, it can not be relied upon as evidence that syphilis does not exist. As the writer has already shown, at least 10 per cent of patients suffering from primary syphilis will give a negative Wassermann reaction, so that, in the presence of a suspicious lesion, the negative reaction is of no value, and the patient should never be assured, upon the strength of such a reaction, that his symptoms are not due to syphilis.

As already emphasized, every suspicious lesion following exposure, whether situated upon the penis or elsewhere, should, if possible, be examined with the dark-field apparatus for *Treponema pallidum.* This should never be neglected and a Wassermann test made in preference, for the dark-field examination is the diagnostic method of choice during this stage of the disease, but where such an examination can not be made, or where it gives negative results, a Wassermann test should always be performed. The writer has observed many instances in which it was impossible to demonstrate *Treponema pallidum,* either by the dark-field apparatus or in stained preparations made from the suspicious lesion, but in which the Wassermann test was positive and thus the diagnosis was established. In extraurethral chancres the test has been of the greatest value in enabling us to make an early diagnosis and in the latent period, after the healing of the initial lesion and be-

fore the appearance of secondary symptoms, the Wassermann test is the only method available for making a diagnosis. It is in this latter class of cases, where there is only a history of a healed lesion to assist one, that the test is of the very greatest clinical value in diagnosis and every such case should be tested repeatedly, if possible, as treatment applied before the appearance of secondary symptoms is much more efficient than when it is administered afterward.

The Interpretation of the Results of the Wassermann Test in the Secondary Stage of Syphilis.—In the secondary stage of syphilis a positive result is obtained in from 95 to 96 per cent of all cases, so that in this stage of syphilis the Wassermann test may be stated to be almost always confirmatory of the clinical findings. However, from 4 to 5 per cent of secondary cases, in the writer's experience, will give a negative reaction, even when repeatedly tested, and when marked secondary lesions are present, so that the mere absence of a positive reaction is no proof that the patient is not suffering from the disease.

A *double-plus* (*four-plus*) reaction in patients presenting the lesions of secondary syphilis or lesions that are suspicious of this stage of the disease, is conclusive evidence of the presence of syphilis under the limitations already mentioned in discussing this same type of reaction in the primary stage; i. e., if the conditions which sometimes give a positive reaction can be eliminated in the particular case examined.

A *plus* (*two-* or *three-plus*) reaction is not of as much value in the secondary stage of syphilis as in the primary, but even in this stage this type of reaction occurs in about 10 per cent of cases, in the writer's experience, and when accompanied by suspicious symptoms, should be interpreted as a positive reaction. In the majority of such instances the reaction is equal to the three-plus reaction reported by most laboratories, inhibition of hemolysis being almost complete, but even if the reaction is less than

this it should be regarded as confirmatory of the presence of the disease in patients presenting clinical symptoms or a clear history of a primary lesion.

A *plus-minus* (*plus* or *plus-minus*) reaction in the secondary stage of syphilis is valueless from a diagnostic standpoint. In these cases the diagnosis will have to rest upon the clinical symptoms present or upon the history of the case. On the other hand, the presence of this type of reaction should not lead to any hope that the disease is not present, as some secondary infections never give more than a plus-minus reaction.

A *negative* reaction when suspicious symptoms of secondary syphilis are present is of greater value than at any other stage of the disease, but it should be remembered that practically five per cent of cases of secondary syphilis give a negative reaction and that many of these cases remain negative for weeks and months. It is only when the negative reaction persists for several months and no symptoms of syphilis are present during this time that it may, with justice, be regarded as conclusive evidence of the absence of syphilis. On the other hand, if the case examined gives no history of a primary lesion and the symptoms present are only slightly suspicious, a negative reaction which persists for two or three months may be regarded as proving the absence of syphilis, provided treatment has not been administered in the meanwhile.

The Interpretation of the Results of the Wassermann Test in the Tertiary Stage of Syphilis.—In the tertiary stage of syphilis, when definite tertiary lesions are present, the Wassermann test gives as high a percentage of positive results as in the secondary stage, but there are many cases tested in this stage in which the symptoms are atypical or only slightly suspicious, or in which the nervous system alone is involved, which give weak or negative reactions, so that the total percentage of positive results in this stage of the disease is slightly below that encountered in the secondary stage. In many of these cases, also, spe-

cific treatment has been administered and although not successful in preventing the development of lesions, it may have reduced markedly the strength of the Wassermann reaction.

A *double-plus* (*four-plus*) reaction with the test in the tertiary stage of syphilis is diagnostic of the disease under the limitations already noted in discussing the interpretation of this type of reaction in the primary and secondary stages.

A *plus* (*two-plus* or *three-plus*) occurs more frequently in this stage of syphilis than in the secondary stage and is, therefore, of greater diagnostic value. In patients presenting suspicious lesions of this stage, especially in those in which there is apparent involvement of the central nervous system, the writer believes that this type of reaction should be given the same diagnostic value as the double-plus (four-plus) reaction, with the same limitations.

A *plus-minus* (*two-plus* and *plus-minus*) reaction, unless permanent, or treatment has been administered, is of no value in the diagnosis of syphilis during this stage of the disease. In those instances in which there is no history of infection and the symptoms present are merely slightly suspicious, this type of reaction should be considered as negative, provided it persists over two or three months, but in the presence of a clear history of infection, especially if treatment has been administered, a *plus-minus* reaction should not lead one to consider the case as nonsyphilitic.

A *negative* reaction in patients suspected of tertiary syphilis is of no value in excluding the disease unless it persists for several months and symptoms have not developed meanwhile. In the absence of a history of infection and of symptoms which are typical, a negative reaction may be interpreted as excluding syphilis, provided no specific treatment has been administered and it remains negative for a period of three months. In all suspected tertiary cases, when the Wassermann test is negative, a test should be made upon the cerebrospinal fluid.

The Interpretation of the Results of the Wassermann Test in the Latent Stages of Syphilis.—The results of the Wassermann test differ in the early and late latent cases but the difference is not sufficient to justify our classifying the cases into early and late cases, so far as the interpretation of the results is concerned. In the early latent cases, that is, the cases tested during the latent period between the healing of the initial lesion and the occurrence of secondary symptoms, a higher percentage of positive results is obtained than in latent periods occurring later in the disease, and the lowest percentage of positive results is obtained in latent cases in which the period of latency occurs after the development of secondary or tertiary lesions. The percentage of positive results obtained by the writer in latent infection, including both early and late cases, has been 68 per cent, so that in this class of infections one must expect about 30 per cent of negative results. One of the most important reasons for the lower percentage of positive results in latent cases is that the vast majority of these patients have received more or less specific treatment and in most of the cases the test was made in order to determine the result of this treatment. Therefore, it follows that the plus reaction will have more diagnostic value in these treated cases than in other stages of syphilis when treatment has not been given, and that a negative reaction will have less value in excluding the disease.

A *double-plus (four-plus)* reaction occurs less frequently in latent cases than in other stages of syphilis, but when it is obtained it is absolutely diagnostic of the disease under the limitations already mentioned.

A *plus (two-plus* or *three-plus)* reaction, in latent cases, where there is a clear history of infection, should be given the same interpretation as the double-plus (four-plus) reaction, especially if specific treatment has been administered. It is the writer's belief that this type of reaction

in latent cases, under the conditions named, is diagnostic of syphilitic infection.

A *plus-minus (plus* or *plus-minus)* reaction in latent infections, when there is a clear history of infection, symptoms have been present in the past, or specific treatment has been given, should arouse the suspicion that the disease is still present, but the test should be repeated two or three times, with a similar result, before a diagnosis of syphilis should be entertained. In such cases a plus-minus reaction usually means insufficient treatment, and indicates that further treatment is required. In the absence of a history of infection, or of previous symptoms, a diagnosis of syphilis should never be made upon a plus-minus reaction.

A *negative* reaction in cases suspected of being in the latent stage of syphilis has no value as excluding the disease unless it is consistently negative over several months, in the absence of specific treatment and of symptoms of the disease. A negative result is obtained in at least 30 per cent of latent syphilitics so that it has little value in excluding the disease.

Interpretation of the Results of the Wassermann Test in Syphilitic Disease of the Central Nervous System.—The interpretation of the results of the Wassermann test in syphilitic disease of the central nervous system is considered in Chapter X of this work.

Interpretation of the Results of the Wassermann Test after Specific Treatment.—The interpretation of the results obtained in patients who have received specific treatment is considered in Chapter IX of this work.

General Summary.—From the writer's experience he believes that the following general conclusions regarding the interpretation of the results of the Wassermann test are justified:

1. If the diseases, other than syphilis, that sometimes give a positive result with the Wassermann test, can be excluded, a double-plus (four-plus of many writers) reaction is absolutely diagnostic of syphilis. It is believed

that, under such conditions, this type of reaction is specific of the disease, whether symptoms are present or not, or whether there is or is not a history of infection.

2. Under the same conditions, a plus reaction (three-plus or two-plus of many writers) may, in primary, tertiary, and latent infections be regarded as diagnostic, provided there is a clear history of infection, or suspicious clinical symptoms are present. In secondary cases this type of reaction is not diagnostic unless typical secondary symptoms are present. In the absence of either history or symptoms this type of reaction should not be regarded as diagnostic of syphilis.

3. A diagnosis of syphilis should never be made upon a plus-minus reaction *alone*. Many perfectly normal individuals give this type of reaction at times in their blood serum. In latent infections a plus-minus reaction, if persistent, should be followed by further specific treatment.

4. A single negative reaction, where there is no history of infection and where symptoms are not present, is of considerable value as a corroborative sign that syphilis is not present, but where there is any suspicion that the disease may be present it has very little value in excluding syphilis. The experiments already mentioned upon the variation in the complement-binding power of the blood serum of known syphilitics illustrate the truth of this statement, and it is only when a negative reaction is repeatedly obtained over a long period of time that it can be considered as good evidence of the absence of the disease, and even then the spinal fluid should be tested and, if possible, a provocative Wassermann test should be made upon the blood. The same remarks apply to cases that have been given specific treatment, a negative reaction being of no value as indicating a cure of the infection, unless the reaction remains persistently negative over a period of at least one year. In such cases a provocative Wassermann test should be made, and the spinal fluid should be tested. A luetin test

should also be made in certain cases before the patient is discharged as cured.

The interpretation of the results of the Wassermann test must rest very largely with the clinician, for the clinical picture present is often more decisive than is the result of the test, and it is the clinician's place to reconcile the result of the test with the clinical picture, rather than the serologist's. The latter should simply report what actually occurs when the patient's blood serum is tested, without any reference whatever to the clinical history or the symptoms which may be present, and it remains with the clinician to interpret the report in the light of his patient's condition. The writer believes that much harm has been done by the serologist allowing the history of a case, or the symptoms present, to influence his reading of the Wassermann test, and it is always better to have the test read without the serologist knowing anything whatever about the history or symptoms of the patients from whom the specimens were collected.

The Wassermann Test as an Index of the Prevalence of Syphilis

Since the discovery of the Wassermann test it has been largely used in determining the prevalence of syphilis in communities, hospitals, and among various classes of people, and has been of the greatest service in demonstrating how widespread this disease really is, and the comparatively large number of people who suffer from it in nearly all countries and among nearly all classes of society. In fact, it may be stated, that before the days of the Wassermann reaction, although it was generally recognized that syphilis was a very prevalent disease in many countries, we had a very poor conception of its real prevalence, especially in the United States, so that from a sociological standpoint, the test has been of such value as to almost revolutionize our ideas regarding the subject.

It is now well known, and has been demonstrated time and again by Wassermann surveys of the class mentioned, that between 80 and 90 per cent of prostitutes who have pursued their calling for as long as five years or more, are syphilitic, although it should be remembered, in considering this high percentage, that only a comparatively small percentage of the women were actually capable of transmitting the infection at the time the tests were made, so far as clinical evidence was obtainable. However, in a disease like syphilis that has its periods of infectivity and quiescence, this high percentage of syphilitics among prostitutes furnishes evidence enough of the danger of illicit sexual intercourse, and one often wonders why the disease is not much more common than it appears to be in the usual professional experience. As a matter of fact, syphilis is much more common, as shown by the results of the Wassermann test, than usually supposed and, in this country as well as in others, is pretty well distributed through all classes.

As a result of the application of the Wassermann test in Wassermann surveys, as they are called, a great mass of evidence has been accumulated regarding the prevalence of syphilis in different communities and among different classes of the population. Thus, Southard[92] performed 6,000 Wassermann tests in the Harvard Neuropathologic Laboratory and found 23 per cent positive; Hammond,[93] who applied the test to both patients and others at the New Jersey State Hospital, found 6.3 per cent positive; Thompson[94] made 1,000 tests at the Arkansas State Hospital for Nervous Diseases and found 33 per cent positive; Rosenberger[95] made 5,106 Wassermann tests upon the patients at the Philadelphia General Hospital, during 1916, and found 27.4 per cent positive; and Johnson,[96] who examined by means of the Wassermann test 224 children showing anemia and malnutrition, found that over 33 per cent gave a positive Wassermann reaction.

There is no better place to study the prevalence of syph-

ilis than among the personnel of the army, for recruits are drawn from all classes of society for the service and from all parts of the United States. The most important and conclusive contribution to our knowledge of the prevalence of syphilis in the army, and, consequently, in civil life, has been made by Lieut. Colonel Vedder, who published his results in 1915,[97] and from whose work I shall quote largely in considering this subject.

In his work, Vedder considered that all cases giving a double-plus (four-plus) reaction were without doubt syphilitic, while those giving a plus (three-plus reaction) were probably syphilitic. Summarized, his results are presented in Table XXXIV:

TABLE XXXIV

OCCURRENCE OF SYPHILIS IN ARMY, FROM VEDDER

CLASSES	TOTAL TESTED	KNOWN CASES %	WASSERMANN %	UNDOUBTED SYPHILITICS %	WASSERMANN %	ESTIMATED TOTAL NUMBER OF PROBABLE SYPHILITICS %
Recruits	1,019	0	7.75	7.75	9.02	16.77
Cadets	621	0	2.57	2.57	2.89	5.46
White enlisted	1,577	3.44	4.77	8.21	7.87	16.08
Colored enlisted	1,472	1.08	21.80	22.21	13.11	36.00
Porto Rico Regt.	531	13.55	28.58	42.37	13.55	55.93
Military convicts	1,145	6.48	9.50	15.98	5.67	21.65
Insane soldiers	567	3.51	8.29	11.80	7.41	19.21
Tuberculous soldiers	229	7.56	15.72	23.28	15.72	39.00
Soldiers home	1,171	11.62	13.40	25.02	9.73	34.75

There may be some question as to whether the cases giving a plus reaction examined by Vedder should be called probably positive, but, even if these cases are excluded, the figures given in the table are very striking and show that syphilis is a far more prevalent disease than is generally supposed by the average practitioner.

Among the recruits examined by Vedder, numbering 1,019, a double-plus (four-plus) reaction was obtained in 7.75 per cent, or practically 8 per cent. These men had

passed a most thorough physical examination for admission to the army and presumably presented no evidences of syphilis when accepted. If to this number be added the 9.02 per cent of cases giving a plus (three-plus) reaction, the total percentage of syphilitics would amount to 16.77 per cent. These recruits were distributed through the areas of the United States covered by the recruiting stations at Fort Slocum, New York, and Columbus Barracks, Ohio, and represented numerous occupations before enlistment. Vedder concludes that his observations, together with the known figures for rejection for syphilis of applicants for admission, show that practically "among young men in civil life between the ages of 20 and 30, and of the general class belonging to the occupations mentioned, the percentage of syphilis may be estimated at at least 16.77 per cent, and there is good reason for believing that it is fully 20 per cent."

The writer's own observations upon the percentage of positive Wassermann reactions obtained in recruits for the army, numbering several thousands of cases, is practically the same as Vedder's, as regards the occurrence of double-plus reactions, the percentage obtained by the writer being 8.64 per cent instead of 7.75. It is not believed that the cases giving a plus reaction should be considered as positive unless there is a history of infection or of suspicious symptoms in the past; but in the writer's experience such a history may be obtained upon careful questioning in enough of the cases to raise the percentage of syphilis among recruits of the army to 10 per cent when they are admitted to the service. If to these are added the percentage of rejections for syphilis, based upon actual symptoms of the disease, the writer believes that at least 15 per cent of all applicants for enlistment for the army, prior to the present war, were infected with syphilis, and this means that practically 15 per cent of the average male civilian population of this country between the ages of 20 and 30 are infected. This appears to be an enormous

percentage but the figures are borne out in Wassermann surveys upon a similar class of men, as regards age, in other countries, and in surveys made in our large hospitals upon all classes of cases admitted to them.

Vedder's results upon the class of young men who have entered West Point are important, for these young men come from the average middle class family of this country, have been well educated, and must be above the average in intelligence in order to pass the rigid examinations for entrance to the military academy. In this class of young men Vedder found 2.57 per cent of double-plus (four-plus) reactions among the 621 cadets tested, and 2.89 per cent of plus reactions. If these be added together, there is a total percentage of over 5 per cent which he considers as probably syphilitic. The writer believes that probably three per cent would be about the amount of syphilis in this class of cases, and, applied to a similar class of young men in civilian life, (the class attending our colleges) it would mean that about this percentage of college men are syphilitic before entering college.

As regards the amount of syphilis in white and colored enlisted men in the army, Vedder found that among the white enlisted men 8.21 per cent gave a double-plus (four-plue) reaction while among the colored enlisted men no less than 22 per cent gave this type of reaction. His figures as regards the greater percentage of syphilitic infection among the colored men of the army agree with those of many investigators who have made Wassermann surveys upon the colored race in this country.

Vedder's results as to the prevalence of syphilis among the patients at the army hospital for tuberculosis, at Fort Bayard, N. M., are of interest, as they demonstrate that a much larger proportion of tuberculous patients give a positive reaction than patients suffering from other diseases. He found that no less than 23.28 per cent of the 229 patients examined gave a positive reaction and he states that his results are almost identical with those of

Letulle, Bergeron, and Lepine in Paris, who found that one out of every five tuberculous patients was also syphilitic. These results explain fully the large number of positive reactions which some writers have claimed are due to tuberculosis, but the most careful investigations have shown that tuberculosis *per se* does not give a positive reaction with the Wassermann test, so that those patients who do react positively are undoubtedly suffering from a coincident syphilitic infection.

The writer, while working upon his method of complement fixation in the diagnosis of tuberculosis, had the opportunity of testing 166 patients in the General Hospital at Fort Bayard and found that nearly 10 per cent of them gave a positive reaction with the Wassermann test, while about 6 per cent were known to be syphilitic, giving a total percentage of 16 per cent of syphilitics in the number examined. While these figures are not as large as those recorded by Vedder they amply demonstrate the fact that syphilis and tuberculosis are more often associated than are other diseases, and that one may expect a large percentage of syphilitic infection among the tuberculous.

Irvine,[98] as the result of various Wassermann surveys, concludes that from 10 to 15 per cent of the population of the State of Minnesota are syphilitic, or from 200,000 to 300,000 people. He states that Wassermann surveys made in the asylum at Fergus Falls showed that 30 per cent of the inmates were syphilitic and that the average of many similar surveys is 20 per cent. Peterson[99] found that among 477 obstetric patients, no less than 5.8 per cent were syphilitic, and of 606 gynecologic patients the test was positive in 8.2 per cent. Regarding the value of the Wassermann test in obstetric and gynecologic practice he says: "In many of the cases, both where there was a history of infection and when this was lacking, physical examination in the gynecologic clinic failed to arose suspicion of the presence of syphilis. The point to be emphasized is that the same careful physical examinations have

been made in this particular clinic for years with only an occasional patient suspected of being luetic. When, however, such examinations are reinforced by routine Wassermann examinations, nearly 10 per cent of the patients are found syphilitic.'' Ladd[100] found that 25.5 per cent of patients applying for treatment at the dispensaries of the Washington, D. C., Casualty and Asylum Hospitals gave a double-plus (four-plus) Wassermann reaction, and that of these 14.6 per cent were colored, and 10.9 per cent white patients.

An entire volume could be written covering the statistics of the almost innumerable Wassermann surveys that have been made in different localities but the illustrations here given demonstrate the value of the test in the study of the prevalence of syphilis. In fact, no longer is any data of scientific value regarding this subject unless it is based upon the results of carefully performed Wassermann reactions, for the day has passed when clinical evidence alone is to be considered as sufficient upon which to base figures regarding the prevalence of syphilis in a community or country. The cases of syphilitic infection that are apparently without clinical symptoms far outnumber those that present typical symptoms and the only way that we at present possess of making a diagnosis in these latent infections is by means of the Wassermann test. Aside from the value of this test in the diagnosis of the disease and in the scientific control of its treatment, perhaps the greatest value of the test is in the demonstration of the extent to which this insidious disease has permeated society, an extent altogether unsuspected before the use of the reaction in Wassermann surveys.

CHAPTER IX

THE EFFECT OF TREATMENT UPON THE WASSERMANN REACTION. THE WASSERMANN TEST AS A CONTROL OF THE TREATMENT OF SYPHILIS. THE PROVOCATIVE WASSERMANN REACTION

The treatment of syphilis, either with mercury or with salvarsan or neosalvarsan often has a marked effect upon the results of the Wassermann test, rendering strongly positive reactions negative or greatly lessening the strength of the reaction in those cases in which a positive result is not rendered negative. This fact has rendered the use of the test invaluable as a guide to the efficiency of the treatment of the disease with various drugs and has enabled the profession to discard many drugs vaunted as specifics for the disease but which have been proved by the Wassermann test to be absolutely worthless. One of the most striking examples of this fact is the instance of sodium cacodylate, which at one time was announced as almost the equal of salvarsan in the treatment of syphilis, but which was soon proved by the Wassermann test to be practically worthless, so far as curing the infection, and which is not used at the present time in the treatment of the disease except by those ignorant of the work which has been done proving its worthlessness or those willfully blind to the results of that work.

The Wassermann reaction is generally conceded to be the most reliable and delicate of all of the symptoms of syphilis and for this reason the effect of treatment upon the reaction is of the utmost practical importance. A posi-

tive reaction, as has been noted, means the presence in the body of living treponemas, and as long as the reaction remains positive, just so long is the patient a victim of syphilitic infection, regardless of the occurrence of other symptoms of the disease. The effect of treatment with various drugs can be easily followed in the case of syphilis by frequently repeated Wassermann tests, and in this way one can secure a reliable picture of the results of any particular form of treatment. In view of the importance of this subject the following chapter contains the personal observations of the writer upon the effect upon the Wassermann reaction of treatment with salvarsan and mercury, which have been previously published,[101] together with remarks upon the test as a control of the treatment of syphilis, and upon the provocative Wassermann reaction.

The Effect of Treatment upon the Wassermann Reaction

Prior to 1911 there was little in the literature upon the effect of treatment on the Wassermann reaction but since that time a great deal of data has been published showing that the reaction is markedly influenced by treatment with certain drugs, and that positive reactions often become negative and remain so for considerable periods of time after the cessation of treatment. Unfortunately, investigation has also shown that the early treatment with mercury and salvarsan was insufficient and that most of the cases so treated relapsed within a year after treatment was stopped, so that at the present time no intelligent practitioner believes that a few injections of mercury or of salvarsan or neosalvarsan will cure syphilis.

The Effect upon the Reaction of Treatment with Salvarsan.—The data given in regard to the effect upon the Wassermann test of treatment with salvarsan was collected by the writer between 1910 and 1913 and is based upon a comparatively few injections of the drug, as Ehrlich's idea

that one or two injections of this drug would sterilize the body, so far as *Treponema pallidum* was concerned, was still the belief and hope of the profession. However, the facts deduced from these experiments and observations have since been fully confirmed and the data demonstrate the effect upon a positive Wassermann reaction of treatment with this drug.

Among those who had contributed valuable data regarding the effect of salvarsan upon the Wassermann reaction prior to the writer's publications may be mentioned the following:

Nichols and Fordyce[102] treated 11 patients with salvarsan with the following results upon the Wassermann reaction: In 8 the reaction became negative, 1 in 19 days; 2 in 21 days; 1 in 23 days; 1 in 25 days; 1 in 39 days; 1 in 3 months; and 1 in 3 months and 11 days. Michaelis[103] treated 110 patients and states that the reaction may become negative within a period from 2 to 10 weeks after the injection. Fordyce[104] states that the results of the Wassermann test after salvarsan have varied within wide limits, in his experience, but in general a change occurred in from 4 to 5 weeks. The earliest change from a positive to a negative reaction observed by him was in 6 days after the injection. He states that "several cases showed alternating negative and positive phases." Noguchi[105] analyzed very carefully 102 cases treated with salvarsan, more than half of them being under observation for over three months while the remainder had been injected four weeks previously. He made quantitative examinations of their blood serum for complement-binding power, the tests being made before the injection of salvarsan, and at intervals of one day, three days, one week, two weeks, three weeks, four weeks, six weeks, eight weeks, etc., after the injection. He found that 30 cases became negative, 24 reduced to less than 1 antibody unit, while the remaining 48 cases still contain more than 1 antibody unit, giving strong positive reactions. In 40 per cent of the primary cases, in 37 per

cent of the secondary cases, in 35 per cent of tertiary, in 33 per cent of latent, in 14 per cent of hereditary, and in 50 per cent of incipient tabes the reaction became negative. The average of the negative reactions equalled 33.7 per cent of the total 102 cases. Of the negative cases reported by Noguchi, 34 in number, 3 became negative in two weeks, 10 in three weeks, 11 in four weeks, 5 in five weeks, 4 in six weeks, and 1 in seven weeks.

Animal Experiments.—The effect upon the Wassermann reaction of treatment with salvarsan can be shown in rabbits experimentally infected with syphilis. Through the kindness of Major H. J. Nichols, the writer had the opportunity of repeatedly testing the blood serum of rabbits experimentally infected with syphilis and yaws, and Tables XXXV and XXXVI illustrate the gradual increase in the strength of the Wassermann test in these animals after infection, and the disappearance of the positive reaction after

TABLE XXXV

RESULTS OF WASSERMANN TEST BEFORE AND AFTER TREATMENT WITH SALVARSAN IN RABBIT INFECTED WITH SYPHILIS*

AMOUNT OF BLOOD SERUM, C.C.	DATE OF TESTS							
	Jan. 23	Jan. 27	Jan. 30	Feb. 6	Feb. 8	Feb. 10	Feb. 13	Feb. 18
0.05	–	+	+	++	–	+	0	–
0.10	–	++	++	++	+–	++	–	–
0.15	0	++	++	++	+–	++	0	–
0.20	++	0	++	++	+–	++	–	–

**Treponema pallidum* found in testicular lesion Jan. 19. 0.02 gram salvarsan per kilo administered intravenously Feb. 6.

TABLE XXXVI

RESULTS OF WASSERMANN TEST BEFORE AND AFTER TREATMENT WITH SALVARSAN IN RABBIT INFECTED WITH YAWS*

AMOUNT OF BLOOD SERUM, C.C.	DATE OF TESTS							
	Jan. 23	Jan. 30	Feb. 2	Feb. 6	Feb. 8	Feb. 10	Feb. 13	Feb. 17
0.05	–	–	++	++	–	–	–	–
0.10	–	+–	++	++	–	+–	–	–
0.15	–	+	++	++	+–	+	–	–
0.20	–	++	++	++	+–	++	–	–

**Treponema pertenue* found in lesion Jan. 21. 0.02 gram salvarsan per kilo administered intravenously Feb. 6.

treatment with salvarsan. The sign (++) indicates complete inhibition of hemolysis.

In the rabbit infected with syphilis the treponema was found in the lesion on January 19, but the Wassermann test did not become positive, even with 0.2 c.c. of the serum, until February 6. One week after the injection of salvarsan the reaction had become negative and the table shows that it had begun to diminish in strength two days after the injection.

The results of treatment with salvarsan upon the rabbit infected with yaws were quite similar, the reaction becoming negative one week after the intravenous injection of the drug.

The following summary of the writer's observation upon the effect of treatment with salvarsan on the Wassermann reaction embraces 500 cases which were observed and tested for at least four months after treatment. Of these 500 cases only 288 became negative, or 57.6 per cent, but these results were due to the prevalence, at that time, of giving but one or two intravenous injections of salvarsan instead of several, the intramuscular method of using the drug giving altogether the best results as regards the disappearance of the positive reaction.

The real effect of treatment with salvarsan upon the Wassermann reaction can only be accurately understood if several factors are taken into consideration, the most important being the stage of syphilis during which the drug is administered; the intensity of the positive Wassermann reaction before treatment; the method of administration of the drug and the dose administered; as well as the amount and kind of previous mercurial treatment.

The Stage of Syphilis in Relation to the Disappearance of the Reaction.—The results of the writer have shown that regardless of the method of administration or the dose, the stage of the disease has much to do with the effect of treatment with salvarsan upon the Wassermann reaction. This is shown in Table XXXVII.

TABLE XXXVII

THE RESULTS OF TREATMENT WITH SALVARSAN UPON THE WASSERMANN TEST IN THE VARIOUS STAGES OF SYPHILIS

STAGE OF DISEASE	TOTAL CASES	BECAME NEGATIVE		REMAINED POSITIVE	
		NUMBER	%	NUMBER	%
Primary	95	63	66.3	32	33.6
Secondary	285	169	59.3	116	40.7
Tertiary	52	17	32.6	35	67.3
Latent	68	39	57.3	29	42.6
TOTALS	500	288	57.6	212	42.4

An analysis of this table is interesting. In 95 primary cases 63 became negative, or 66.3 per cent; in 285 secondary cases, 169, or 59.3 per cent became negative; in 52 tertiary cases 17 or 32.6 per cent became negative; and in 68 latent cases, including both early and late latent infections, 35, or 57.3 per cent became negative.

From the above it is evident that the effect upon the Wassermann reaction of treatment with salvarsan is most marked during the primary stage of the disease, as would be expected and least marked during the tertiary stage, and that the longer the infection has lasted the less is the effect upon the reaction of the treatment.

Time of Disappearance of the Reaction in Relation to the Stage of Syphilis

The rapidity with which the positive Wassermann reaction disappears after treatment with salvarsan depends largely upon the stage of the disease as is shown in Table XXXVIII.

TABLE XXXVIII

THE RELATION OF THE STAGE OF SYPHILIS TO THE TIME OF DISAPPEARANCE OF THE WASSERMANN REACTION, AFTER TREATMENT WITH SALVARSAN

STAGE OF DISEASE	TOTAL CASES	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	5 WEEKS	6 WEEKS	7 WEEKS	8 WEEKS
Primary	63	6	25	20	8	2	2	0	0
Secondary	169	13	44	39	33	17	13	5	5
Tertiary	17	3	6	2	5	0	1	0	0
Latent	39	2	9	7	10	6	5	0	0
TOTALS	288	24	84	68	56	25	21	5	5

Regarding the time of disappearance of the reaction after treatment with salvarsan it will be noted that the best results were obtained in the primary stage of the disease, no less than 51 of the 63 primary cases that became negative becoming so within three weeks and the longest time after treatment in which the reaction became negative in this stage was six weeks. In the secondary stage the reaction disappeared generally during the second, third, or fourth weeks, the longest period being eight weeks. In the tertiary stage the results appear more favorable than in the primary, 1 of the 17 cases becoming negative within three weeks but the number of cases is too small to base such an assertion upon, and the writer believes that the evidence is sufficient to prove that the best results are obtained in the primary stage of syphilis.

As will be noted, none of the reactions, in any stage of the disease, became negative after eight weeks, so that it would appear that a positive reaction which remains so after a period of eight weeks has elapsed since the last salvarsan treatment, should always be an indication for further treatment. If the drug is given intravenously, as it generally is today, the period may be shortened to less than this, but it is believed that eight weeks is a practical and safe period to wait in order to ascertain if the Wassermann reaction will become negative.

The Relation of the Intensity of the Reaction to Its Disappearance after Salvarsan.—The stronger the Wassermann reaction the less is it affected by treatment with salvarsan, as is shown in Table XXXIX. In this table and those that follow, it should be remembered that a double-plus (++) reaction means absolute inhibition of hemolysis, or the four-plus reaction of most writers; a plus (+) reaction at least 50 per cent of inhibition, or the three- and double-plus reaction of some laboratories; and a plus-minus, less than 50 per cent of inhibition.

An analysis of this table shows that of 295 cases in which the Wassermann reaction was double-plus (four-plus),

TABLE XXXIX

THE RELATION OF THE INTENSITY OF THE WASSERMANN REACTION TO ITS DISAPPEARANCE AFTER TREATMENT WITH SALVARSAN

CHARACTER OF REACTION	TOTAL CASES	BECAME NEGATIVE		REMAINED POSITIVE	
		NUMBER	%	NUMBER	%
++	295	145	41.9	150	50.8
+	169	110	65.0	59	34.9
+−	36	33	91.6	3	8.3
	500	288	57.6	212	42.4

41.9 per cent became negative; of 169 giving a plus (three or two plus) reaction, 65 per cent became negative, and of 36 cases showing a plus-minus reaction, 91 per cent became negative. As regards the latter cases it may be stated that they were either very early primary cases or cases that had previously received specific treatment to a greater or lesser extent. It will thus be seen that the poorest results as regards the effect of treatment upon the Wassermann test were obtained in cases showing complete inhibition of hemolysis and the results gradually grew better with the loss in the strength of the reaction.

The Relation of the Stage of Syphilis and the Intensity of the Wassermann Reaction to its Disappearance after Treatment with Salvarsan.—The percentage of negative results obtained by treatment with salvarsan in each stage of syphilis is considerably influenced by the intensity of the reaction. Table XL illustrates the effect of the intensity of the reaction upon its disappearance in each stage of the disease.

From this table it is evident that the effect of treatment with salvarsan upon the Wassermann test, in every stage of syphilis, varies with the intensity of the reaction, being most pronounced in the cases showing the weaker reactions and least in those giving complete inhibition of hemolysis. From these data it is evident that the earlier treatment is begun in syphilis the better will be the results, for the cases of tertiary syphilis, for instance, which gave a double-plus reaction, gave a very low percentage of negative re-

TABLE XL

THE RELATION OF THE STAGE OF SYPHILIS AND THE INTENSITY OF THE WASSERMANN REACTION TO ITS DISAPPEARANCE AFTER TREATMENT WITH SALVARSAN

STAGE OF SYPHILIS	CHARACTER OF REACTION	TOTAL CASES	BECAME NEGATIVE		REMAINED POSITIVE	
			NUMBER	%	NUMBER	%
Primary (95 cases)	++	44	24	54.5	20	45.4
	+	41	31	75.6	10	24.3
	+−	10	8	80.0	2	20.0
Secondary (285 cases)	++	185	98	52.9	87	47.0
	+	86	58	67.4	28	32.5
	+−	14	13	92.8	1	7.1
Tertiary (52 cases)	++	33	5	15.1	28	84.8
	+	16	9	56.2	7	43.7
	+−	3	3	100.0	0	0.0
Latent (68 cases)	++	33	18	54.5	15	45.4
	+	26	12	46.1	14	53.8
	+−	9	9	100.0	0	0.0

sults after treatment; i. e., only 15 per cent and even in the cases giving a plus reaction the percentage of negative reactions was only a little over 50 per cent. It will be observed that in the latent stage a higher percentage of cases showing a double-plus reaction became negative than of those showing a plus reaction, but this was due to previous mercurial treatment in a larger proportion of the cases giving a double-plus reaction.

From these data it is also evident that the intensity of the Wassermann reaction in the various stages of syphilis is of considerable prognostic importance. While in all of the stages of syphilis, with the exception of the latent stage, the results were better in cases giving a plus reaction than in those giving a double-plus, the difference is most marked in the tertiary stage of the disease and least in the primary. As regards the double-plus cases, the best results were obtained in the primary and latent stages of syphilis and the poorest results in the tertiary stage. The good results in the latent stage may be explained by the fact that many of these cases were early latent cases, for the late latent cases are much more resistant to treatment. Of the cases giving a plus reaction the best results were obtained in

the primary stage, the poorest in the latent stage, while the results in the secondary stage were better than those in the tertiary. The prognosis, as regards the disappearance of the reaction, is most unfavorable in the tertiary cases giving a double-plus (four-plus) reaction.

The Relation of the Method of Administration of Salvarsan to the Disappearance of the Wassermann Reaction in Treated Cases.—The method of administration of salvarsan has a very marked influence upon the effect of the drug on the Wassermann reaction. Of the 500 cases studied, 209 were treated by the intramuscular injection of the alkaline solution, 249 by the intravenous injection of the drug, and 42 by combined intramuscular and intravenous injections. Table XLI illustrates the effect of the method of administration upon the Wassermann reaction.

TABLE XLI

THE METHODS OF ADMINISTRATION OF SALVARSAN IN RELATION TO THE DISAPPEARANCE OF THE WASSERMANN REACTION IN TREATED CASES

METHOD OF ADMINISTRATION	TOTAL CASES	BECAME NEGATIVE		REMAINED POSITIVE	
		NUMBER	%	NUMBER	%
Intramuscular	209	159	76.0	50	24
Intravenous	249	101	44.5	148	55.5
Combined	42	28	66.6	14	33.3
TOTALS	500	288	57.6	212	42.4

It will be observed, from a study of the table, that the effect of intramuscular injections of salvarsan upon the reaction is much more pronounced than other methods of administration. Unfortunately, this method of administration has been almost abandoned by the profession and in the army it has been entirely replaced by the intravenous method, owing to the pain and complications that followed the injections and the time lost in hospital. However, there is no question that the intramuscular method is infinitely more efficient in treatment than the intravenous, as shown by the results upon the Wassermann test, for of the 209 cases so treated, most of them receiving but one injection of 0.6 gram of salvarsan, 159, or 76 per cent became

negative, and, as will be noted later, in discussing the test as a control of treatment, fewer cases treated by the intramuscular method relapsed than by the intravenous.

The figures given for the intravenous method are below what they should be for the reason that the majority of the cases studied only received one intravenous injection. Further observations upon this method of administration and its effect upon the Wassermann reaction have shown that with from three to five intravenous injections the results are as good as those obtained with the intramuscular injections, but the data here given are sufficient to show that the Wassermann reaction can be rendered negative by only one intravenous injection in at least 40 per cent of the cases.

The effect upon the reaction of the combined intramuscular and intravenous method of using salvarsan was determined in 42 cases, of which 66 per cent became negative, thus indicating that the results were not as good with the combined method as with the intramuscular method alone. However, this result was due to the fact that a number of the intramuscular cases had two injections of salvarsan and some three, and it is considered that one intramuscular injection of the drug is equal to at least three intravenous injections so far as the effect upon the Wassermann test is concerned.

As would be expected the effect of treatment with salvarsan upon the reaction increases with the number of doses of the drug that are administered, regardless of the method of administration. Of 200 cases receiving one intramuscular injection of salvarsan of 0.6 gm., 152, or 76 per cent became negative, while of 9 cases receiving two intramuscular injections, 7, or 77.7 per cent became negative. There is little difference between these two groups of cases so far as the apparent effect upon the Wassermann test is concerned, but the number of cases receiving two intramuscular injections is too small to allow of our basing any accurate statistics upon, as a larger

number of cases would undoubtedly give a higher percentage in this class of cases.

Of the cases treated by the intravenous method, 177 were given one intravenous injection of 0.6 gm. of salvarsan, and 72, or 40.6 per cent became negative; 52 were given two intravenous injections of the same dose, of which 22, or 42.3 per cent became negative; while 10 cases were given three intravenous injections, of which 7, or 70 per cent became negative. There was but little difference in the percentage of negative results obtained in those given one and two intravenous injections, and it will be noted that two intravenous injections did not have as much effect upon the Wassermann reaction, as one intramuscular injection. The percentage of negative results obtained with three intravenous injections, however, approaches closely to that obtained with one intramuscular injection, and justifies the assertion that one intramuscular injection is equal, in its effect upon the Wassermann reaction, to three intravenous injections of salvarsan.

The time of the disappearance of a positive reaction after treatment with salvarsan varies with the method of administration as shown in Table XLII.

Table XLII

The Relation of the Method of Administration of Salvarsan to the Time of Disappearance of the Wassermann Reaction in Cases Treated with Salvarsan

METHOD OF ADMINISTRATION	TOTAL CASES	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	5 WEEKS	6 WEEKS	7 WEEKS	8 WEEKS
Intramuscular	159	9	36	39	31	18	18	4	4
Intravenous	101	13	44	24	16	4	0	0	0
Combined	28	2	4	5	9	3	3	1	1

From the table it will be noted that the positive reaction disappeared more slowly after intramuscular injections of the drug than after the intravenous injections. After intravenous injections there were only four cases that became negative during the fifth week after injection, and none after that period, while no less than 26 cases

treated by the intramuscular method became negative after the fifth week. The cases treated by the combined method were also slower in becoming negative than those treated by the intravenous method alone.

If the Wassermann reaction is due to the presence in the tissues of living treponemas, as is now generally accepted, one would expect that a positive reaction would disappear more rapidly after an intravenous injection of salvarsan than after an intramuscular injection. The drug, when administered intravenously, is quickly brought into contact with large numbers of the organisms and they are killed, while those not immediately affected and which are accessible to the drug perish later, but as the drug is eliminated within a few days it is obvious that what action it has occurs comparatively quickly and if the reaction is dependent upon the treponemas and they are destroyed rapidly, the reaction also should disappear within a comparatively short period of time. On the other hand, when salvarsan is administered intramuscularly it is absorbed very slowly and exerts its destructive action upon the treponemas for days and weeks, a smaller number are killed in a given period of time, and therefore, the reaction persists for a longer period than after intravenous administration. Whether the positive Wassermann reaction is due to substances liberated from the treponema during life; produced by the tissues in answer to the irritation caused by the treponema multiplying within them; or to substances liberated from the organism after its destruction or to the reaction of the tissues to such substances, is immaterial, so far as the disappearance of the reaction after the administration of salvarsan is concerned, for, however produced, it is evident that the reaction theoretically should disappear more rapidly after intravenous injection than after intramuscular, and this is what actually occurs in practice.

The Effect upon the Reaction of Treatment with Mercury.—The Wassermann test demonstrated the true value of mercury in the treatment of syphilis by the fact that

this drug is capable of causing the disappearance of a positive reaction, but it has also demonstrated the fact that it is of comparatively small value when compared with salvarsan. For one case that becomes negative after treatment with mercury there are hundreds that become negative after treatment with salvarsan, and although the majority of both classes of cases relapse in time and again present a positive Wassermann reaction, the relapses are much less frequent among patients treated with salvarsan than with mercury.

The relative efficiency of salvarsan and mercury in causing a disappearance of the Wassermann reaction is well illustrated in patients who had previously been treated with mercury before receiving salvarsan. Of 90 patients who were treated with mercury by the mouth for nine months or more before the administration of salvarsan and who gave a positive Wassermann reaction, 68, or 70.5 per cent became negative within eight weeks after the administration of the drug. The intensity of the reaction at the time of receiving salvarsan and the length of time the patients had been taking mercury is given in Table XLIII.

Table XLIII

The Wassermann Test in Patients Who Had Taken Mercury for Various Periods of Time

Method of Treatment	Time of Treatment	Number of Cases	Character of the Reaction		
			++	+	+−
Mercury by mouth	9 months	17	8	7	2
" " "	1 year	26	16	8	2
" " "	2 years	17	7	9	1
" " "	3 years	8	3	4	1
Totals		68	34	28	6

The table shows that 26 of these patients had taken mercury by mouth for a period of one year and that 16 of them still gave a double-plus (four-plus) reaction; that 17 had received the same treatment for two years, of whom 7 still gave a double-plus reaction; and that 8 had received

treatment for three years, of whom 3 still gave a double-plus reaction. Of the 68 cases not one had become negative as the result of mercury administered by the mouth, but after the administration of salvarsan every one became negative within 8 weeks. This is certainly very decisive proof of the greater specific action of salvarsan and could only be ascertained by the effect of the drugs in question upon the Wassermann reaction.

Objection may be raised to the results recorded above by calling attention to the fact that the administration of mercury by the mouth is now well recognized to be the very poorest of all ways of giving this drug, although it is not so long ago that three years' treatment by mouth with mecury was considered by the best authorities upon the disease as adequate for the cure of syphilis. In answer to this objection Table XLIV is given, covering 18 patients treated with hypodermic injections of mercury, a plan of treatment generally acknowledged to be the most efficient of the many ways of administering the drug. For purposes of comparison the effect upon the Wasserman of treatment with salvarsan is also included.

TABLE XLIV

THE COMPARATIVE RESULTS OF TREATMENT WITH MERCURY AND SALVARSAN UPON THE WASSERMANN REACTION

NUMBER OF CASES	*Mercurial Treatment*		*Salvarsan Treatment*	
	INJECTIONS OF GREY OIL	CHARACTER OF REACTION	DOSE 0.6 GM.	CHARACTER OF REACTION
2	7	++	Intramuscular	–
1	8	+	" "	–
1	9	++	" "	–
1	11	++	" "	–
3	15	++	Intravenous	–
1	15	+	Intramuscular	–
4	18	++	" "	–
2	19	++	Intravenous	–
1	20	++	" "	–
1	25	+	Intramuscular	–
1	30	+	" "	–

From a consideration of this table it is evident that cases having had as many as 18 to 20 injections of grey oil

still gave a double-plus Wassermann reaction and that cases having as high as 25 and 30 injections still gave a plus (three-plus) reaction. After the administration of salvarsan all became negative within 8 weeks, and in 12 of the cases only one intramuscular injection of 0.6 gm. of salvarsan was administered. While there is reason to believe that previous treatment with mercury may have had something to do with the good effects produced by salvarsan upon the reaction, it must be admitted that the results obtained in these cases demonstrate beyond all question the superior specific value of salvarsan upon *Treponema pallidum.*

Patients who have been previously well treated with mercury show a higher percentage of negative results after treatment with salvarsan than those who have received no mercurial treatment. Thus of 75 patients who had received mercurial treatment before receiving salvarsan, 84 per cent became negative, while of 110 patients who had received no treatment before the administration of salvarsan, only 74.5 per cent became negative.

In order to illustrate the persistence of the positive Wassermann reaction after treatment with mercurials Table XLV has been prepared giving the method of treatment, the length of time the patient was under treatment, the number of cases observed, and the intensity of the reaction.

The table illustrates very well the effect of treatment with mercury upon the Wassermann reaction, as it occurs in the usual routine of serological work in a Wassermann laboratory. It shows that continued treatment with the drug for long periods of time has but little effect upon the reaction in the majority of instances and that interrupted treatment continued for many years does not produce a negative reaction in many cases. It should not be thought, however, from the data given here, that the Wassermann reaction never becomes negative after treatment with mercury, for in a considerable proportion of cases it does, and

TABLE XLV

ILLUSTRATING THE PERSISTENCE OF THE WASSERMANN REACTION AFTER TREATMENT WITH MERCURY

METHOD OF TREATMENT	LENGTH OF TREATMENT	NUMBER OF CASES	INTENSITY OF THE REACTION		
			++	+	+–
By mouth	1 month	2		2	
" "	2 months	3	1	2	
" "	3 "	4	2	2	
" "	5 "	6	3	3	
" "	6 "	10	7	1	2
" "	7 "	4	3	1	
" "	8 "	5	4	1	
" "	9 "	10	7	1	2
" "	10 "	3	2	1	
" "	11 "	3	3		
" "	1 year	24	13	6	5
" "	14 months	2	2		
" "	15 "	2	1	1	
" "	16 "	4	3		1
" "	17 "	1	1		
" "	18 "	7	1	4	2
" "	19 "	4	3	1	
" "	20 "	2	1	1	
" "	2 years	15	8	5	2
" "	2.5 "	3		2	1
" "	3 "	5	1	2	2
" "	4 "	1	1		
By mouth*	7 "	1		1	
By mouth*	10 "	1	1		
By mouth*	12 "	1		1	
Inunctions	2 months	2	2		
"	5 "	1		1	
"	6 "	2	2		
"	1 year	1	1		

*Interrupted treatment during this time. ++ indicates complete inhibition of hemolysis.

the writer has observed many cases in which a negative Wassermann reaction was obtained after from one to two years proper treatment with this drug but it has been his experience that most cases becoming negative after mercurial treatment relapse in the course of months or a year or two. While he would not go so far as to state that the Wassermann test never becomes permanently negative after treatment with mercury, it has been his experience that it is only in very rare instances that a permanently negative result is obtained from treatment with this drug alone, no matter how it is administered or in what dosage. In fact, it has been proved in rabbits experimen-

tally infected with syphilis, that before the animals can be rendered sterile as regards *Treponema pallidum* so much mercury has to be administered as to cause either the death of the animal or very serious pathological lesions due to the drug. In the writer's rather large experience he has never personally followed a case treated with mercury alone, in which the Wassermann test was positive before treatment, that became permanently negative, although many cases have lost the positive reaction for a while but have invariably become positive again upon the cessation of treatment. However, that mercury does cure syphilis in some instances, is proved by the fact that individuals are encountered who undoubtedly had the disease but who have been without symptoms for many years and who give a negative Wassermann reaction whenever tested.

If the patient's blood serum be carefully titrated after the administration of either salvarsan or mercury, it will be found that in almost every instance the Wassermann reaction is influenced to some extent, although it may never become negative. Thus, many cases showing a double-plus reaction, or absolute inhibition of hemolysis, with quantities of blood serum as small as 0.02 c.c. will become negative for this amount of serum, although giving a positive reaction with the usual quantity of serum used in the routine tests; i. e., 0.1 c.c. By titrating the inhibitory strength of each patient's serum it will be found that, although the Wassermann test may apparently be uninfluenced when the diagnostic amount of serum is used, that is, 0.1 c.c., some diminution in the strength of the reaction will be observed in amounts less than 0.1 c.c. In using the Wassermann test as a control of treatment the titration of the patient's serum is of great importance, as will be noted.

Not only will treatment with salvarsan or mercury markedly influence the strength of the reaction but if treatment is commenced early in the primary stage of the disease it will sometimes prevent the development of a positive reaction, although clinical symptoms may occur. The writer

has observed several such instances, in which secondary symptoms developed in spite of treatment, but in which a positive Wassermann reaction was never obtained.

The Wassermann Reaction as a Control of Treatment

Aside from its value as a diagnostic agent, the greatest value of the Wassermann test for syphilis is found in its use as a guide to the treatment of the disease. It will be evident, from what has been said of the effect of treatment upon the positive reaction that, if scientifically used, the efficacy of any form of treatment advocated for syphilis may be absolutely determined by employing the test as a control of that treatment. In the army the test has been of the greatest practical value in ascertaining the relative efficiency of the various methods of treating syphilis and it has been very largely used for this purpose. As an illustration of the extent of the use of the reaction in the army in the control of the treatment of syphilis, the writer may say that he has personally made almost twenty thousand Wassermann tests upon treated patients, all of these tests being for the purpose of controlling the treatment of the disease, and his experience has been that of all other medical officers who have had charge of the army laboratories where the Wassermann test is performed.

By means of the Wassermann test, repeated at frequent intervals, one is able to diagnose a relapse of syphilitic infection long before the appearance of clinical symptoms, and in this way enabled to treat the infection before it has resulted in such symptoms, thus saving the patient much anxiety and discomfort, and obviously at a stage when it is much more amenable to treatment than after gross clinical symptoms of relapse have occurred. The writer believes that the first symptom of a relapse of a syphilitic infection, in the vast majority of cases, is the recurrence of a positive Wassermann reaction, and that this recurrence often happens weeks and even months before marked clinical

symptoms are noted. In other words, the positive Wassermann reaction is the most delicate symptom of relapse in syphilis that is known, a symptom that occurs long before definite clinical symptoms, and thus it is of the greatest value as an indication of the need of further treatment in cases where it has once disappeared and then recurs. For this reason all cases of syphilis that have been given specific treatment should be tested at frequent intervals in order to ascertain the effect of the treatment upon the reaction, and after a negative reaction is once obtained a test should be made at least every three months for a period of at least one year, when, if the reaction is still negative, a provocative Wassermann test, a Wassermann test upon the spinal fluid, and a luetin test should be made, before the patient is pronounced cured.

The writer has already mentioned the great importance of titrating the complement-binding power of the syphilitic patient's blood serum in judging of the effect of any treatment upon the Wassermann reaction and in using the test as a control of treatment, and the method of making such titrations. The titration of the patient's blood serum should be done whenever it is possible, but especially in those cases that show a continued double-plus (four-plus) reaction after specific treatment has been administered for some time. In such cases, also, the spinal fluid should be tested if the Wassermann on the blood shows no diminution when the serum is titrated, for it is in this class of cases that early involvement of the central nervous system is most apt to have occurred.

The data derived from the use of the Wassermann test as a control of treatment has enabled us to use both mercury and salvarsan intelligently and has added greatly to our knowledge of the action of other drugs that have been advocated from time to time in the treatment of the disease. It has demonstrated beyond all doubt that the absence of clinical symptoms is no proof of cure in this

disease, even though symptoms may be absent for months or years, and that a single negative Wassermann reaction is of absolutely no value in determining either the question of the existence of syphilis or of its cure.

Perhaps the most striking proof of the value of the Wassermann test in the control of the treatment of syphilis is the results that have been obtained by numerous workers upon the occurrence of relapse after treatment with salvarsan, as shown by the Wassermann test. In 1913, the writer published[106] certain observations upon relapse in syphilis after treatment with salvarsan and has continued collecting data upon this subject which have confirmed the observations published. They were undertaken for the purpose of determining the relation of relapse to the dose and method of administering the drug but serve equally well to illustrate the value of the Wassermann test in controlling the treatment of syphilis and in ascertaining the therapeutic results following treatment with a specific drug. Used in this manner the Wassermann test has furnished the following information regarding the occurrence of relapses after treatment with salvarsan:

Four hundred cases were selected for study, and were observed for a period of eight months or more. Of the 400 cases, 202, or 50.5 per cent remained positive after the administration of the drug, while 198, or 49.5 per cent became negative to the Wassermann test after salvarsan was given. Of the 198 cases that became negative, 172, or 86.8 per cent have relapsed; i. e., the Wassermann test has again become positive.

The Stage of Syphilis in Relation to Relapse.—The relation of the stage of syphilis to relapse following treatment with salvarsan is shown in Table XLVI.

A consideration of the table shows that 88.5 per cent of the primary cases relapsed, as shown by the Wassermann test; 84.3 per cent of the secondary cases; 88.8 per cent of the latent; and only 69.5 of the tertiary cases. The latter had all received considerable treatment with mercury prior

TABLE XLVI

THE STAGE OF SYPHILIS IN RELATION TO RELAPSE AFTER TREATMENT WITH SALVARSAN

STAGE OF DISEASE	NUMBER OF CASES	NUMBER OF RELAPSES	% OF RELAPSES
Primary	35	31	88.5
Secondary	115	97	84.3
Tertiary	23	16	69.5
Latent	25	22	88.8

to the administration of salvarsan, so that the comparatively low percentage of relapses in this stage undoubtedly was due to this fact, for the writer believes that in untreated cases the tertiary stage would show as large, and probably a larger, percentage of relapses than either the primary or secondary. The latent stage showed the largest percentage of relapses and this was expected, as it is this class of cases that most often prove resistant to treatment, so far as the disappearance of the Wassermann test is concerned, but the difference is only slight, as shown by the table.

The Relation of the Intensity of the Wassermann Reaction to Relapse.—The cases giving the strongest Wassermann reaction furnished the largest percentage of relapses, as is shown in Table XLVII. Here, as in all tables in this work, the sign, ++, indicates complete inhibition of hemolysis, the four-plus reaction of many authorities:

TABLE XLVII

THE RELATION OF THE INTENSITY OF THE WASSERMANN REACTION TO RELAPSES AFTER TREATMENT WITH SALVARSAN

CHARACTER OF REACTION	NUMBER OF CASES	NUMBER OF RELAPSES	% OF RELAPSES
++	110	104	94.5
+	88	68	77.2
	198	172	86.8

This table demonstrates that of the 110 cases giving a double-plus reaction, no less than 104, or 94.5 per cent relapsed, while of the 88 cases giving a plus (three-plus

or two-plus) reaction, only 68 or 77 per cent relapsed. Therefore it is evident that the stronger the Wassermann reaction the greater the number of relapses following treatment with salvarsan.

Time of Relapse after Treatment with Salvarsan.—By controlling the treatment of syphilis with salvarsan it has been possible for us to obtain accurate data regarding the time of relapse after treatment with salvarsan; i. e., the period elapsing from the time the Wassermann test became negative to the time in which the test again became positive. The data accumulated show that, in the majority of instances, a Wassermann relapse, as it may be called, occurs within eight months after cessation of treatment. Thus, of the 172 cases that relapsed after treatment with this drug, no less than 159 cases relapsed within eight months after treatment. Table XLVIII gives the time of relapse after treatment observed in these cases.

TABLE XLVIII

THE TIME OF RELAPSE AFTER TREATMENT WITH SALVARSAN AS SHOWN BY THE WASSERMANN TEST

TIME OF RELAPSE	NUMBER OF CASES	TIME OF RELAPSE	NUMBER OF CASES
1 month	8	5 months	17
5 weeks	7	6 "	12
6 "	5	7 "	5
7 "	6	8 "	5
2 months	38	9 "	2
9 weeks	1	10 "	2
10 "	7	11 "	1
11 "	2	12 "	2
3 months	26	13 "	1
14 weeks	1	15 "	1
4 months	19	22 "	1
		24 "	1

From this table it will be noted that no less than 38 cases relapsed at the end of two months and 26 at the end of three months, so that it would appear that these periods are of special significance as regards the time of relapse. It is also interesting to note that no less than 100 of the 172 cases that relapsed did so by the end of three months and no less than 149 of the 172 cases by the end of six

months after cessation of treatment. From this it would appear that the vast majority of cases of syphilis treated as these were, with salvarsan, will relapse within six months so far as the Wassermann reaction is concerned, and that it is during this period that repeated Wassermann tests are most essential.

The statement that has already been made regarding the worthlessness of a single negative Wassermann reaction is well illustrated by this table, and even in those cases in which the reaction remains negative for a long period of time, no assurance can be given that it will not again become positive, for it will be observed that some of these cases had a negative Wassermann reaction for as long as one and two years and became positive.

As regards the *time of relapse in the various stages of syphilis* it may be stated that the Wassermann tests showed that the average time of relapse in primary cases was 4½ months; in the secondary cases, 4 months; in the tertiary cases, 3 months; and in the latent cases, 3½ months. The latent cases included both early and late latent cases, which accounts for the longer period of relapse in this class. As will be noted, relapses occur earlier the longer the syphilitic infection has lasted.

The Relation of the Method of Administration of Salvarsan to Relapses.—The Wassermann test has shown that the percentage of relapses after salvarsan varies considerably with the method of administration of the drug. The three methods that have been tested in this way have been the intramuscular injection of the drug, the intravenous injection, and the combined intramuscular and intravenous injection. Table XLIX gives the number of cases treated which have been thus tested and the number of relapses, together with the percentage of relapse in each class of cases.

The number of cases tested that were treated by the combined intramuscular and intravenous method was so small that the percentage of relapses; i. e., 66.6 per cent, can not

TABLE XLIX

THE RELATION OF THE METHOD OF ADMINISTRATION OF SALVARSAN TO RELAPSE, AS SHOWN BY THE WASSERMANN TEST

METHOD OF ADMINISTRATION	TOTAL NUMBER OF CASES	RELAPSES	
		NUMBER	%
Intramuscular	74	54	72.9
Intravenous	115	106	92.1
Combined intramuscular and intravenous	9	6	66.6

be considered as a fair estimate of the percentage that would relapse were a greater number of cases tested. However, the number of cases tested that were given salvarsan intramuscularly and intravenously is large enough to base fair estimates upon, and it is very evident that a much smaller number of relapses follow the intramuscular method of administering the drug than the intravenous method. In justice to the intravenous method it should be stated that fully 50 per cent of the cases tested had only received one dose of the drug, but even after two injections the results were not as good as after one intramuscular injection. Of 80 cases receiving one intravenous injection of 0.06 gm. of salvarsan 78 relapsed, or 97.5 per cent, while of 67 cases receiving the same dose intramuscularly, 51 relapsed, or 76 per cent.

From what has been stated regarding the study of relapse in syphilis after the administration of salvarsan some idea may be gained regarding the great value of the Wassermann test when it is used as a control of treatment or as a test of the efficiency of any drug recommended in the treatment of the disease. The results that have followed the modern method of treatment; i. e., numerous intravenous injections of salvarsan combined with mercurials, given in the proper manner, are far superior to those which have been noted in this chapter with either drug alone. There is but one way of determining what these results are and that is by the use of the Wassermann test as a control of the treatment of the disease. Without it, the physician is at as great a disadvantage in judging of

the results of his treatment of syphilis as the mariner would be without a compass, and this test may well be called the compass of the physician in the treatment of this disease.

The Interpretation of the Reaction when the Test is used in the Control of Treatment.—A Wassermann test should not be performed for the purpose of controlling the treatment of syphilis until treatment with salvarsan, mercurials, or other agents has been omitted for at least a month and the test should be repeated at monthly intervals regardless of the reaction if treatment is not again instituted. If the reaction is negative monthly tests should be made in all cases possible. If it remains persistently negative treatment should not be persisted in, but if there is any trace of inhibition beyond a weak plus-minus reaction, the treatment should be continued. *It should always be remembered that a single negative reaction is absolutely valuless as indicating the cure of the infection* and repeated tests must be made with a similar result before any opinion favoring cure is warranted. Too many patients have been assured that they were cured of syphilis on the strength of one or two negative Wassermann reactions, with disastrous results to innocent individuals, as well as to the patient, and the utmost caution should be exercised in expressing any opinion regarding ultimate cure in syphilis. It should be remembered that the Wassermann test has shown that the vast majority of treated syphilitic infections eventually relapse, even though the treatment has been scientific and thorough, so that the prognosis in every case should be very guarded.

In primary infections which have been treated and in which the Wassermann test has become negative, a return of the reaction even to the extent of a plus-minus reaction, when it is persistent, should indicate further treatment. In the secondary cases a plus-minus reaction is not of as much importance, as most of these cases will give a stronger reaction if they relapse, but even in such cases

if the plus-minus reaction persists for several weeks, treatment should be instituted. In tertiary and latent cases the occurrence of a plus (two- or three-plus of some authors) reaction after treatment has been stopped should be followed by the resumption of the treatment.

Treatment in syphilis should never be discontinued until the Wassermann test has become negative unless it is found that the case is "Wassermann fast"; i. e., that no amount of treatment will cause the reaction to become negative. Wassermann fast cases are frequently met with in practice and occur during every stage of the disease but such cases occur more frequently in tertiary and latent infections than in primary or secondary. In the "Wassermann fast" cases the clinical symptoms, aside from the positive Wassermann reaction, may, and generally do, disappear under treatment, but the reaction never becomes negative.

The Provocative Wassermann Reaction

In 1910, Gennerich[107] called attention to the fact that cases presenting a negative Wassermann reaction often showed a positive reaction after the administration of a dose of salvarsan, this reaction occurring either within a few hours after the injection or within two weeks following it. He called attention to the value of this phenomenon in the diagnosis of obscure syphilitic infections, and as a method of determining whether a patient is really cured of the infection. He suggested that this so-called "provocative Wassermann" be used in treated cases that had presented negative reactions for some time, and were presumably cured, as, in such instances he had found the provocative test frequently positive, thus demonstrating that the disease was still present. Milian,[108] of Paris, about the same time, called attention to the value of the procedure in the study of the disease, as well as Herxheimer,[109] and Michaelis,[110] but to Gennerich belongs the

credit of first actually applying the test in the diagnosis of syphilis and as a control of the treatment of the disease.

Method of Making a Provocative Wassermann Test.—The writer recommends the following procedure in making a provocative Wassermann test: The suspected patient is given from 0.3 to 0.6 gm. of salvarsan intravenously and the blood is collected and tested 12 hours after the administration of the drug; 24 hours after administration; and every day for at least ten days. If the negative reaction is to become positive, it generally does so within from a few hours to 5 or 6 days after the salvarsan has been given, but delayed reactions have occurred not infrequently up to 10 days after administration, and it is good practice to make a test upon the 12th and 14th days after administration, in addition to those recommended. During the first two days after salvarsan has been given, if it is possible, the blood should be collected at intervals of three or four hours and tested, as sometimes the reaction is very evanescent in character and quickly disappears. However, owing to the labor involved in making so many tests it is seldom practicable to do this, and the procedure first mentioned will generally prove sufficient to detect the reaction, if it occurs. Stokes and O'Leary[111] recommend that the blood be drawn at the time of injection and daily thereafter for one week, and that all blood collected be tested with several antigens at one sitting and with the same reagents, in order to avoid any difference in the results that might occur from variations in the reagents used in the test from day to day.

Results of the Test.—In a recent contribution, King,[112] as the result of his investigation of the provocative test upon a very small number of patients, came to the conclusion that the provocative Wassermann reaction does not exist, but that the positive result that sometimes follows an injection of salvarsan is due either to normal variations in the complement-binding power of syphilitic serum, as shown by the writer, or to variations in the technic or

reagents used in the test when made upon different days. His work is very far from convincing and the united experience of hundreds of observers who have used the provocative test in their laboratories and practice is unanimous as regards the actual occurrence of this phenomenon and as to the value of the test in the diagnosis of syphilis and the control of its treatment.

Nichols,[113] after stating that it is a well-established fact that in a syphilitic a negative Wassermann reaction can be converted into a positive one by an injection of salvarsan, gives the following table illustrative of the effect of the provocative test in ten patients in whom a luetin test was also performed at the same time. Table L gives the results that he obtained in these cases:

TABLE L

RESULTS OF THE PROVOCATIVE WASSERMANN TEST AND THE LUETIN TEST. (AFTER NICHOLS.)

CASE NO.	TIME ORDINARY WASSERMANN REACTION HAD BEEN NEGATIVE	LUETIN TEST	PROVOCATIVE WASSERMANN TEST
1	16 months	–	–
2	21 "	–	–
3	24 "	–	–
4	24 "	–	–
5	24 "	–	–
6	15 "	+	++
7	17 "	+	++
8	18 "	+	++
9	19 "	+	++
10	?	+	++

This table is conclusive proof of the occurrence of the provocative reaction, as it shows complete agreement between the results of this test and the luetin test, for it is hardly probable that, if the positive reaction in these cases was due to variation in the complement-binding power of the blood serum or inaccuracies in technic, the results of the provocative test would be identical with the results of the luetin test in every case.

Nichols calls attention to Case 10, in the above table, be-

cause of the interesting results obtained as regards a delayed provocative reaction. The patient was infected in 1906 and had two years of treatment by the mouth with mercury and potassium iodide. No symptoms had occurred since 1908 and in March, 1913, he gave a negative reaction. On April 18th he was given an intravenous injection of 0.6 gm. of salvarsan and his blood was tested every day with the following results: April 18th, negative; April 19th, negative; April 20th, negative; April 21st, negative; April 22nd, negative; April 23rd, double-plus (four-plus).

This case well illustrates the value of the provocative reaction in patients who have had no symptoms of syphilis for several years and who give a negative Wassermann test.

Stokes and O'Leary,[114] in a very excellent study of the provocative Wassermann reaction, obtained very significant results. They made 103 provocative tests, of which 19, or 18.4 per cent resulted in a reversal of the ordinary Wassermann from negative to positive. This small percentage of positive results with the test is not unusual and it has been the writer's experience that it is seldom that a higher percentage than 20 per cent is obtained with the provocative Wassermann test upon limited groups of cases. If thousands of tests were made it is probable that the positive percentage would be somewhat higher but from 18 to 20 per cent of positive results is about all that can be expected from the reaction.

As to the character of the cases showing a positive reaction with the test, Stokes and O'Leary state that 26.3 per cent presented no clinical symptoms of syphilis, but that the remainder show suspicious symptoms although the Wassermann was negative. However, they state that "In no one of the positive cases were the signs so indubitable that the provocative could be regarded as unnecessary or merely confirmatory." They say further: "we believe the test has distinct value in determining the status of a patent under treatment."

Stokes and O'Leary give Table LI showing the results

obtained with the provocative Wassermann test in different types of syphilis.

TABLE LI

EFFICIENCY OF THE PROVOCATIVE TEST IN VARIOUS TYPES OF SYPHILIS

TYPE	NUMBER OF CASES	% POSITIVE
Heredosyphilis	2	0
Osseous	5	20
Central nervous system	12	25
Vascular	3	33
Latent	10	40
Late cutaneous	5	60
Late mucous membrane	5	80

While they admit that the number of cases treated is not large enough to base accurate conclusions upon, they state that, from their series, it appears as though the provocative test is least useful in the class of cases where it is most needed, but, even if this proves to be true, and we can only expect 20 per cent of positive results in osseous syphilis, 25 per cent in syphilis of the central nervous system, and 33 per cent in vascular syphilis, the writer believes that the test is well worth while, as often it will be the only method available for making a diagnosis in this class of cases.

The indications for a provocative Wassermann reaction are given by Stokes and O'Leary in their paper and are so admirable that this section of the paper is here quoted:

"1. A definite history of primary or secondary lesions or a suspicious genital sore of any description. (With a negative Wassermann.)

"2. Syphilis in husband or wife or a history of a sore in either.

"3. Treated cases to determine the fact of cure or need for further treatment. One-third of the cases thus tested by us, gave a positive provocative effect.

"4. Obscure bone or joint lesions.

"5. Histories of miscarriages unless the anatomical cause is glaringly obvious.

"6. Mothers of syphilitic children without clinical signs of the disease.

"7. Cases with a history of a positive Wassermann elsewhere, negative on present examination.

"8. Mental deviates and constitutionally inferior individuals with suspicious histories.

"9. Certain signs elicited by special examinations, such as decreased bone conduction with normal hearing, chorioretinitis and retinitis pigmentosa, bilateral dacryocystitis in childhood," etc.

In all the above conditions the writer agrees with the authors that a provocative Wassermann test should be made, if possible, for in nearly all cases in which the indications for the test cited have occurred, the writer has found the test of service.

As an illustration of the results obtained with the test in cases of syphilis that have not shown symptoms for a long period of time and in which the ordinary Wassermann test has been negative for months and even years, Table LII is given:

TABLE LII

RESULTS OF PROVOCATIVE WASSERMANN TEST IN SYPHILIS

CASE NO.	TIME SINCE TREATMENT	TIME SINCE WASSERMANN BECAME NEGATIVE	RESULT OF WASSERMANN REACTION	RESULT OF PROVOCATIVE REACTION
1	12 months	12 months	–	++
2	13 "	13 "	–	–
3	14 "	14 "	–	++
4	15 "	15 "	–	++
5	16 "	16 "	–	–
6	17 "	17 "	–	++
7	18 "	18 "	–	++
8	19 "	19 "	–	++
9	20 "	20 "	–	++
10	21 "	21 "	–	–
11	24 "	24 "	–	–
12	24 "	24 "	–	++
13	24 "	24 "	–	–
14	25 "	25 "	–	++

The cases given in the above table are selected from a large number because they show the results of the provoc-

ative reaction month after month, as regards time, and because the data relating to them is of such a nature as to make the test definite and conclusive. It will be seen that a positive reaction was obtained as long as 25 months after the cessation of treatment, during which time no symptoms of syphilis had been noted and the Wassermann reaction was always negative, when tested.

The writer is firmly convinced, from personal experience, that a dose of salvarsan or neosalvarsan will frequently convert a negative Wassermann reaction into a positive one and that this phenomenon is not due to any variation occurring normally in the patient's blood serum or to differences in technic or in the reagents used in making the test. When one has tested cases week after week, always with a negative result, and when these same cases after a dose of salvarsan or neosalvarsan become positive within a few hours or days after the administration of the drug, there can be only one conclusion, and that is that the positive result has been produced by the drug, and the writer has had this experience too many times to allow of the least doubt in his mind of the existence of a provocative reaction following the administration of these drugs. It is true that the test often fails in cases in which one would expect it to be positive, but this is no proof of the nonexistence of the phenomenon.

Cause of the Provocative Reaction.—The exact nature of the provocative reaction is still undetermined. There are two theories that have been discussed in this connection, the first being that the reaction is due to the stimulation of the treponemas by the drug and the consequent production of more of the substance or substances which give rise to a positive Wassermann reaction; while the second is that the drug kills sufficient of the treponemas to increase the amount of toxin and thus the syphilitic antibody, as it may be called, is increased. The first theory depends upon the belief that *Treponema pallidum,* during its life and multiplication in the tissues causes the production of

some substance which produces the Wassermann reaction, and that a small dose of salvarsan stimulates the few remaining organisms in the negative Wassermann cases to overproduction of this substance for a short time, and thus the negative Wassermann reaction becomes positive. The second theory rests upon the belief that the organisms killed by the drug either liberate the substance producing the positive reaction, or a toxin which stimulates the production of this substance in the tissues of the body. Neither theory has been proved correct as we know nothing certain as to the actual cause of the Wassermann reaction.

Some authorities have recommended intramuscular injection of mercury for the production of the provocative Wassermann reaction but the reports concerning the efficacy of this method are so conflicting that it is not recommended.

The provocative Wassermann test is a useful adjunct to our diagnostic methods for syphilis and should be resorted to whenever it is possible, both in the diagnosis of the disease and in determining the question of the cure of the infection. The large amount of work involved in making the numerous tests that are necessary in correctly using the provocative reaction will always operate to limit its use to comparatively few cases but whenever it is possible to use the test it should be employed.

From the evidence that has accumulated it may be stated that *in patients who have presented a negative Wassermann reaction for long periods of time and in whom symptoms of syphilis have been absent during this period, the provocative Wassermann test often results positively. The same result is sometimes obtained in cases presenting suspicious symptoms but in which the ordinary Wassermann test is negative, or in cases which give a history of infection but with no symptoms and a negative Wassermann reaction.*

CHAPTER X

THE WASSERMANN TEST UPON THE CEREBROSPINAL FLUID. INTERPRETATION OF RESULTS. THE COLLOIDAL GOLD TEST. THE CELL COUNT AND TESTS FOR INCREASE IN GLOBULINS IN THE CEREBROSPINAL FLUID

Recent observations have shown the great importance of the Wassermann test upon the cerebrospinal fluid and it may be stated that no case of syphilis can be considered as having had the best treatment unless this fluid has been tested, even though the Wassermann test upon the blood is negative and the patient has been free from symptoms for long periods of time. It is now demonstrated beyond doubt that involvement of the central nervous system frequently occurs very early in this disease and this demonstration has been rendered possible by the results of the Wassermann test upon the cerebrospinal fluid during the early latent and secondary stages of the infection.

The Wassermann test upon the cerebrospinal fluid often shows involvement of the central nervous system before any clinical symptoms are observed and the use of the test has rendered intensive treatment possible and has resulted in either preventing the development of such symptoms or in greatly delaying their appearance. Formerly it was believed that if the test gave a negative reaction in the cerebrospinal fluid and a positive one in the blood, the diagnosis of cerebrospinal syphilis should be made, the result serving to distinguish this condition from paresis, in which both the blood and cerebrospinal fluid usually gave a posi-

tive reaction. However, it has since been shown that the negative result in the cerebrospinal fluid depended entirely upon the amount of the fluid used in the test and that if a large enough amount was used a positive result could be obtained in the fluid in almost 100 per cent of cases of cerebrospinal syphilis. Therefore, it follows, that the Wassermann test upon the cerebrospinal fluid, so far as its value in differentiating various syphilitic conditions of the central nervous system is concerned, depends upon quantitative factors entirely, so that it is necessary in testing this fluid to test different quantities if one is to secure the best results from the test. The importance of the Wassermann test upon the cerebrospinal fluid is shown by the statement of Fordyce[115] that "From 25 to 35 per cent of syphilitic individuals have positive findings in the spinal fluid during the first year of their infection" and during this time symptoms referable to the central nervous system are frequently present. This being so, the importance of ascertaining in every case of syphilis whether the central nervous system is involved at as early a date as possible, can hardly be overestimated, and for this reason the cerebrospinal fluid should be tested as well as the blood serum, although the necessity of the test upon the spinal fluid has not, as yet, been fully realized by the medical profession.

The writer has already described the method of obtaining the cerebrospinal fluid and the technic required (Chapter V) in making the test. As the differentiation of some of the syphilitic diseases of the central nervous system depends upon the quantity of fluid tested, the writer recommends that 0.2, 0.5, 0.7 and 1 c.c. quantities be tested in each case, with his method of performing the reaction, the fluid, of course, not being inactivated before use. The quantity of spinal fluid tested, with any method, should always be at least four times the quantity of blood serum tested with the particular method used, and it is always best to use both smaller and larger amounts than this in

order to determine the strength of the positive reaction upon which depends the differential diagnosis of the condition present.

Hauptmann and Hossli[116] were the first to insist upon the use of large quantities of the cerebrospinal fluid in applying the Wassermann reaction and their work changed entirely the prevalent conception of the comparative rarity of a positive reaction in diseases like tabes and demonstrated that almost any syphilitic disease of the central nervous system would give a positive Wassermann reaction with these larger amounts of fluid. Thus, the finding of a positive reaction in the cerebrospinal fluid, once looked upon as almost pathognomonic of paresis, has ceased to point to this disease unless it is obtained with small amounts of the fluid, for with large amounts other syphilitic diseases of the central nervous system also give a positive reaction.

Because of the use of too small amounts of the cerebrospinal fluid in making the Wassermann test much of our statistical data regarding the occurrence of a positive reaction in the various syphilitic infections of the central nervous system are worthless and a great many divergent results are reported in the literature owing to this fact. Mueller[117] obtained 75 per cent of positive reactions in tabes, and 98 per cent in paresis, while Boas found that all of his untreated cases of tabes gave a positive reaction. This is a very good illustration of the confusion in results due to the use of different amounts of the spinal fluid, for Boas used larger amounts of the fluid in his tabetic cases and obtained a much higher percentage of positive results.

The writer's experience may be summed up as follows:

In *paresis* the Wassermann test is positive in the blood in nearly 100 per cent of the cases, and if 1 c.c. of the cerebrospinal fluid be used, it is positive in practically 100 per cent of the cases. If smaller amounts of the spinal fluid be tested the percentage of positive reactions will be

found to decline, until when but 0.2 c.c. of the fluid is tested only from 75 to 80 per cent of the cases will give a positive reaction. When 0.5 c.c. of the fluid is used practically 98 per cent of cases of paresis will give a positive reaction in the spinal fluid, a result never found in any other syphilitic condition of the central nervous system.

In *cerebrospinal syphilis,* if 1 c.c. of the spinal fluid be tested almost 100 per cent of the cases will prove positive, but if only 0.2 c.c. of the fluid be tested only about 10 per cent will prove positive. The Wassermann on the blood in these cases gives from 60 to 75 per cent of positive results, as reported by different investigators.

In *tabes,* if 1 c.c. of the cerebrospinal fluid be tested, the Wassermann will be positive in about 95 per cent of the cases, but if only 0.2 c.c. of the fluid be tested the positive results will only amount to from 5 to 10 per cent. In this disease the Wassermann test upon the blood gives about 70 per cent of positive reactions.

From the above it is apparent that in differentiating between paresis, cerebrospinal syphilis and tabes, the accurate titration of the complement-binding power of the cerebrospinal fluid is most important. If 1 c.c. of the fluid is used for the test, the results will be positive in practically all of the cases of either disease, but if smaller amounts of the fluid be used it will be found that results are obtained, which, if taken in conjunction with the clinical symptoms present, will generally enable the correct diagnosis to be made. Thus, if a positive reaction is obtained with 0.2 c.c. of the fluid, or less, the diagnosis is almost certainly paresis, for only from 5 to 10 per cent of cases of cerebrospinal syphilis or tabes give a positive result with this amount of fluid. If a positive reaction is obtained with 0.1 c.c. of the fluid the diagnosis of paresis will be found correct in 99 per cent of the cases examined.

The Test upon the Cerebrospinal Fluid in the Various Stages of Syphilis.—As stated, recent researches have

shown that involvement of the central nervous system in syphilis occurs early in the disease in a considerable proportion of the cases, as has been shown by the results of the Wassermann test upon the cerebrospinal fluid. In fact, so good an authority as Fordyce holds that paresis, tabes, and the majority of other manifestations of syphilis on the part of the nervous system originate "at the time of the general infection in the first year" and there is much to support this theory in the biological and clinical characteristics of syphilitic infection.

Results in Primary Stage.—The writer has no knowledge of a well authenticated positive result of the Wassermann test upon the cerebrospinal fluid in the primary stage of syphilis.

Results in the Secondary Stage.—The older writers upon this subject all agreed in stating that cases showing no symptoms of involvement of the central nervous system, in the secondary stage of syphilis, were invariably negative to the Wassermann test upon the spinal fluid, or to other tests upon this fluid, while, if symptoms of involvement were present the Wassermann was generally positive. One exception may be made to this statement in the case of Ravaut[119] who, in 1903, reported that among 116 cases in which the spinal fluid was tested, no less than 67 per cent showed some abnormality of the fluid, either in an increased cell count or an increase in globulins. The more recent investigators have reported numerous instances in which the Wassermann test upon the cerebrospinal fluid was positive early in the secondary stage, even when there were no symptoms of involvement of the central nervous system. Thus Wile[120] found 30 per cent of the cases of secondary syphilis that he tested gave a positive result with the Wassermann test upon the spinal fluid, and Craig and Collins[121] found that a considerable proportion of their early secondary cases gave a positive Wassermann reaction in the spinal fluid. Ellis and Swift[122] examined 22

cases of untreated early secondary syphilis and found the spinal fluid negative in all, but in 56 cases of late secondary syphilis, tested by Altman and Dreyfus,[123] no less than eleven gave a positive Wassermann reaction in the spinal fluid.

Results in the Tertiary Stage.—The results of the Wassermann test upon the cerebrospinal fluid in paresis, cerebrospinal syphilis, and tabes have already been discussed, but it should be remembered that the examination of the fluid in this stage of the disease from many cases in which there are no discoverable lesions in the central nervous system will result positively, as stated by Kolmer.[124] The writer has repeatedly observed cases of tertiary syphilis that gave a positive Wassermann reaction with the cerebrospinal fluid but in which there was absolutely no evidence of involvement of the central nervous system, but he would be loath to conclude from this that no lesions due to *Treponema pallidum* were present. At any rate such a finding is the signal for a very careful scrutiny of the patient and a very guarded prognosis as regards future involvement of the central nervous system.

Results in the Latent Stage.—The results of the test upon the cerebrospinal fluid in the latent stage of syphilis will vary, of course, with the involvement of the central nervous system, but the percentage reported by most observers does not exceed 15 per cent, including both early and late latent infections.

The Interpretation of the Results of the Test

The interpretation of the results of the Wassermann test upon the cerebrospinal fluid, as regards the intensity of the reaction, are the same as already noted in the discussion of the interpretation of the results upon the blood, with this exception; that a positive Wassermann reaction upon the cerebrospinal fluid is specific of syphilis, in the opinion of practically all observers. The writer has never

seen a positive reaction upon the spinal fluid in any other condition than syphilis and his experience is that of all who have studied this phase of the subject.

It is also true that more double-plus (four-plus) reactions are observed in the spinal fluid than in the blood although occasionally weak reactions are noted with the maximum amount of fluid tested. Of course, in cases of tabes and cerebrospinal syphilis, the smaller amounts of fluid show gradations in the strength of the reaction, but a diagnosis of syphilis of the central nervous system should never be made upon anything less than total inhibition of hemolysis; i. e., a double-plus (four-plus) reaction, when as large an amount of the serum as 1 c.c. is tested.

The writer has already given the significance of the positive reaction when obtained with the different amounts of the cerebrospinal fluid tested but will here summarize the interpretation to be given the reaction in this respect:

If amounts of the spinal fluid as small as 0.05 or 0.1 c.c. give total inhibition of hemolysis the diagnosis is almost certainly paresis; if 0.2 c.c. of the fluid give the same result, the diagnosis is, in all probability, paresis; if larger amounts up to 0.5 c.c. of the fluid are used, the diagnosis is probably paresis; while if still larger amounts are used the diagnosis must rest largely upon the clinical symptoms present, as cerebrospinal syphilis, tabes, and paresis all give positive results with the larger quantities of fluid.

In order to illustrate the difference in results obtained by using different amounts of the cerebrospinal fluid, Table LIII is inserted, showing the results of the Wassermann test upon cases of cerebrospinal syphilis, tabes and paresis.

A consideration of this table will show the importance of testing different amounts of the cerebrospinal fluid and how useful such titrations are in the differentiation of the conditions mentioned, especially the differentiation of paresis from cerebrospinal syphilis and tabes.

It will be understood that the interpretation of the results of the Wassermann test upon the cerebrospinal fluid

TABLE LIII

THE RESULTS OF THE WASSERMANN TEST UPON THE CEREBROSPINAL FLUID IN CEREBROSPINAL SYPHILIS, TABES, AND PARESIS

NAME OF INFECTION CASE NO.	WASSERMANN IN BLOOD	AMOUNT OF CEREBROSPINAL FLUID C.C.					
		0.05	0.1	0.2	0.5	0.7	1
Cerebrospinal syphilis							
1	++	–	–	+	++	++	++
2	–	–	–	–	+	++	++
3	++	–	–	–	+–	++	++
4	+	–	–	–	–	+	++
5	+–	–	–	–	+–	++	++
6	++	–	–	+–	++	++	++
7	–	–	–	–	–	++	++
8	+	–	–	–	+	++	++
Tabes							
1	++	–	–	+	++	++	++
2	++	–	–	–	+	++	++
3	–	–	–	+	++	++	++
4	++	–	–	++	++	++	++
5	+	–	–	–	+	+	+
6	++	–	–	–	+–	+	++
Paresis							
1	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++
3	++	+	++	++	++	++	++
4	++	++	++	++	++	++	++
5	++	++	++	++	++	++	++
6	++	++	++	++	++	++	++
7	+	+	++	++	++	++	++
8	++	++	++	++	++	++	++

should be based not only upon this test but also upon the result of the cell count, the colloidal gold reaction and the tests for the increase in globulins, as all should be taken into consideration in arriving at a just conclusion regarding the condition present in any case, but the Wassermann reaction alone is sufficient to base a diagnosis of syphilitic infection of the central nervous system upon, if positive.

In using the Wassermann test upon the cerebrospinal fluid in the determination of the cure of syphilis the reaction should be negative with 1 c.c. of the fluid. If this quantity of the fluid gives even a partial positive result the case should not be considered as cured but treatment should be continued.

The early occurrence of involvement of the central nerv-

ous system, as shown by the results of the Wassermann test, renders this procedure upon the cerebrospinal fluid as essential as upon the blood in syphilitic infections, and the test should be employed much more frequently than it now is as a routine measure. No patient should be assured that he is free from syphilitic infection unless the cerebrospinal fluid has been found to give a negative reaction with quantities as large as 1 c.c., but, on the other hand, a diagnosis of involvement of the central nervous system should not be made unless complete inhibition of hemolysis occurs with this quantity of spinal fluid.

The Wassermann test upon the blood, in cases showing some nerve condition, if positive, does not prove that the condition is syphilitic in nature, but if the cerebrospinal fluid is also positive, the condition is almost certainly syphilitic. While the blood serum is often negative in certain syphilitic conditions of the central nervous system, it is but rarely that the cerebrospinal fluid is negative when quantities as large as 0.7 to 1 c.c. are employed in the test, so that the examination of the spinal fluid will detect many syphilitic infections that would be overlooked if one depended entirely upon the result of the Wassermann test upon the blood. For this reason the writer urgently recommends the testing of the cerebrospinal fluid in every case of syphilis in which it is possible to do so and this test should never be omitted in the determination of the cure of a syphilitic infection. The operation of lumbar puncture, when properly performed, is not dangerous or very painful, and the amount of information that is often secured by the examination of the cerebrospinal fluid more than compensates for any discomfort that may be caused by the collection of the fluid.

The Colloidal Gold Test

The colloidal gold test was recommended by Lange,[125] in 1913, as a method of distinguishing between certain syph-

ilitic and nonsyphilitic diseases of the central nervous system, and today this test stands as one of the most useful confirmatory methods that we possess in the diagnosis of syphilitic disease of this system.

The exact nature of the reaction in the colloidal gold test is unknown. Lange believes that it is a method which enables us to measure the protein content of the cerebrospinal fluid, for he found that this fluid under conditions in which the protein content was increased caused the precipitation of the gold solution and that, within certain dilutions, this precipitation was characteristic of syphilitic conditions of the central nervous system. Previously, Zsigmondy[126] had demonstrated that the clear red color of solutions of colloidal gold, prepared first by him, changed to a blue with the precipitation of the gold upon the addition of electrolytes, and that solutions of protein in the presence of an electrolyte inhibited the precipitate and that it was possible in this way to determine for various proteins the inhibitive point and thus differentiate them. Accordingly, Jaeger and Goldstein[127] regard the reaction obtained in the colloidal gold test as a purely physical reaction of an electrolytic nature, but others, especially Zaloziecki,[128] believe it to be an immunity reaction. It is true that the reaction is obtained when the protein content of the spinal fluid is increased but it is yet too early to insist that it is wholly due to protein increase in the fluid.

The literature concerning this test is now quite voluminous and all investigators are unanimous in their statements regarding the value of the test in syphilis of the central nervous system, but there is considerable disagreement as to the weight to be placed upon the various findings with the test and their exact significance. However, all agree that the test is practically specific for paresis and some of those having a large experience with it insist that a diagnosis of this condition may be made from the result of this test alone. The writer can not agree with this opin-

ion of the value of the test in paresis, although it is undoubtedly true that in at least 96 to 98 per cent of cases the typical paretic curve is obtained, but the same type of reaction has been observed in other conditions, although very rarely. However, the fact that a paretic curve does occur in other conditions than paresis renders it impossible for the test to be considered as absolutely specific of this condition, although it must be regarded as the most valuable of all confirmatory evidences of the presence of the disease.

Technic of the Test.—The technic of the colloidal gold reaction is very simple, but the preparation of the solution of colloidal gold is extremely difficult and the utmost care must be used at every stage of the process or results will not be satisfactory. In fact, it may be said that the entire technic of the test really consists in the preparation of the solution of colloidal gold, for if a good solution is obtained there is little difficulty in performing the test.

Preparation of the Colloidal Gold Solution.—It is absolutely requisite that all chemicals used in the preparation of the solution be chemically pure and the products of Merck have been found most satisfactory by numerous workers. The following chemicals are used in the preparation of the colloidal gold:

1. Merck's Gold Chloride. Acid.
2. Merck's Blue Label Oxalic Acid. Crystals.
3. Merck's Blue Label Potassium Carbonate.
4. Merck's Formaldehyde. Highest Purity, 40%.

All glassware used in making the colloidal gold solution should be thoroughly washed in hot water with ivory soap, rinsed in tap water for several minutes; then with hot bichromate solution, allowing the beakers, pipettes, and test tubes to remain in the bichromate solution for fifteen or twenty minutes; after which they are rinsed in distilled water and finally in triple distilled water. The glassware should be used as promptly as possible after cleaning, the

pipettes and test tubes being dried in a hot air oven before use. Thermometers used in preparing the solution should be cleaned in the same manner as the glassware.

The distilled water used should be distilled in an apparatus in which there are no rubber connections and the water should be distilled three times before it is used. In certain regions, where the water is very heavily impregnated with mineral salts, it may have to be distilled four, or even five times, and this is also true if the water used has been heavily impregnated with chlorine in purifying it for domestic use.

The following method of preparing the colloid of gold solution is recommended by Kolmer[129] who states that it is adapted from the method of Miller, Brush, Hammers, and Felton.[130]

1. Heat 1000 c.c. of triply distilled water over a proper burner in a properly washed beaker containing a thermometer.

2. When the temperature reaches 60° C. add 10 c.c. of a 1 per cent solution of Merck's gold chloride crystals in triply distilled water and 7 c.c. of a 2 per cent solution of Merck's blue label potassium carbonate in triply distilled water.

3. At 80° C., while stirring briskly, add 10 drops of a 1 per cent solution of Merck's blue label oxalic acid crystals, in triply distilled water.

4. At 90° C. remove the burner and, while stirring, add 5 c.c. of a solution of 1 c.c. of Merck's highest purity formaldehyde in 40 c.c. of triply distilled water, or enough to produce an initial pink color.

5. The solution must be neutral in reaction when used and to determine the reaction it is titrated with a 1 per cent solution of alizarin red, in 50 per cent alcohol. With this indicator the neutral point is shown by a brownish red tint; an acid solution gives a lemon-yellow, and an alkaline solution a purple-red, color.

In determining the reaction the following procedure is carried out:

In a properly cleaned beaker is placed 10 c.c. of the colloidal gold solution and two drops of the indicator are added. If a brownish red tint appears the solution is satisfactory; if not, the color is noted, and according to whether it is acid or alkaline it is titrated with either $\frac{N}{50}$ NaOH or $\frac{N}{50}$ HCl. If acid the NaOH is used and if alkaline the HCl. When the neutral point is reached; i. e., a brownish red tint appears, the amount of reagent neces-

sary to neutralize the 10 c.c. of the gold solution is noted and the amount necessary to neutralize 1000 c.c. is readily calculated. Neutralization should be made with normal or decinormal solutions of the acid or alkali, as the case may be.

Kolmer gives the following standards for an efficient colloidal gold solution:

1. It must be absolutely transparent and of a brilliant red orange or salmon red color. (The writer has found that if there is the least trace of cloudiness in the solution, it will be found unserviceable.)
2. Five cubic centimeters of the solution should be completely precipitated in one hour by 1.7 c.c. of a 1 per cent solution of sodium chloride in distilled water.
3. The solution must be neutral in reaction when used.
4. The solution must not produce a reaction greater than a red blue discoloration (No. 1 reaction) with normal cerebrospinal fluid, and should give a typical curve with a known paretic fluid.

To the above the writer would add that the colloidal gold solution should show no evidence of having been deposited on the glass flask in which it is stored or any evidence of a precipitate within the flask. It has been noted by the writer that some glassware is entirely unsuitable for use with colloidal gold as it apparently is attacked by it with a resulting precipitate in the solution or deposited upon the sides and bottom of the storage flask. Jena glassware is the best but Pyrex has been found satisfactory. The colloidal gold should be kept in the flask in the dark and will keep for months unchanged if properly handled.

The fact that the colloidal gold solution is perfectly clear does not indicate that it will prove effective in actual practice for it has been found that perfectly clear solutions are sometimes worthless. Such solutions are called "protected solutions" because no precipitation will take place after the addition of any electrolyte, while "unprotected solutions," as they are called, act efficiently in the test, and are those that can be completely precipitated by 1.7 c.c. of a 1 per cent sodium chloride solution in one hour. This test of the efficiency of a colloidal gold solution is very important and should always be carried out before any

such solution is depended upon for use in the colloidal gold test. It has also been determined that alkaline colloidal gold solutions are practically inert when used with a positive cerebrospinal fluid, while acid solutions give little reaction with a positive fluid, and an abnormal reaction with a normal spinal fluid. If the colloidal gold solution is only slightly acid it will react well with paretic fluids, but will give a reaction with normal fluids similar to that obtained in the luetic zone with fluid from some syphilitic conditions. *In order to secure reliable results the colloidal gold solution must be neutral in reaction.*

Method of Performing the Test.—The technic of the colloidal gold test is very simple but to secure proper results each step must be carefully performed. All glassware must be prepared as already described and pipettes must be perfectly dry before being used.

Eleven test tubes, cleaned as described and perfectly dry, are arranged in a rack and in the first tube is placed 1.8 c.c. of freshly prepared, sterile 0.4 per cent sodium chloride solution prepared from chemically pure sodium chloride. Into each of the ten remaining test tubes place 1 c.c. of the salt solution. With a dry pipette add 0.2 c.c. of the cerebrospinal fluid to be tested, which must be free from blood, to the first tube and mix thoroughly. After mixing, 1 c.c. of the mixture is transferred to the second tube, mixed, and 1 c.c. of this mixture transferred to the third tube, and this process repeated until the tenth tube is filled, when from that tube 1 c.c. is discarded. The eleventh tube acts as a control and will contain no cerebrospinal fluid. The dilutions which have thus been made are as follows: 1-10, 1-20, 1-40, 1-80, 1-160, 1-320, 1-640, 1-1280, 1-2560, and 1-5120.

After the dilutions of the cerebrospinal fluid have been made, as indicated, there is added to each tube 5 c.c. of the colloidal gold solution. This is thoroughly mixed with the fluid in the tubes and the rack kept at room temperature

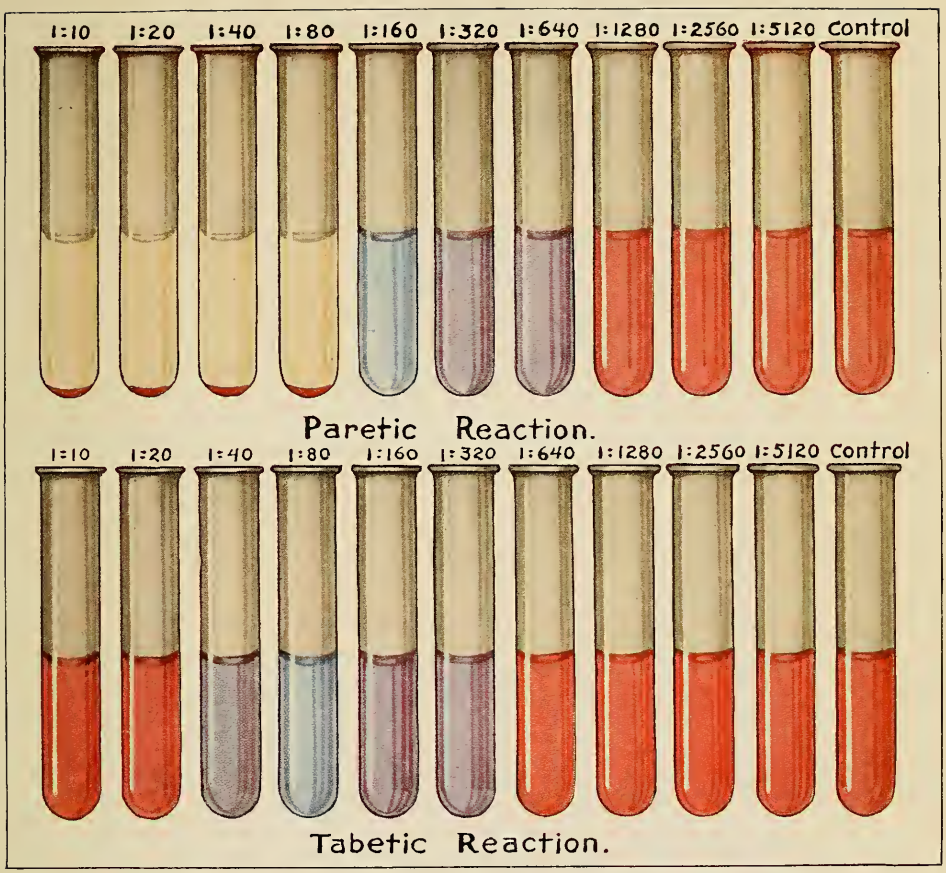

Plate III.

Paretic Reaction.—The upper row of tubes illustrates the reaction, or curve, obtained with the colloidal gold test in the cerebrospinal fluid of paresis and is known as the paretic curve or reaction.

Tabetic Reaction.—The lower row of tubes illustrates the reaction, or curve, obtained with the colloidal gold test in the cerebrospinal fluid of tabes and is known as the tabetic reaction. (Luetic-zone curve.)

overnight, the test being read upon the next day and recorded by the numbers corresponding to the changes in the color of the mixture observed, as follows:

5. Complete precipitation. The supernatant liquid is colorless.

4. Pale blue.

3. Blue.

2. Lilac or purple.

1. Red-blue.

0. No change.

Reading the Results.—The results of the test are read by the numbers corresponding to the color changes, as noted in the preceding table. Thus, if the first four tubes show complete precipitation, with a colorless supernatant fluid, the fifth and sixth tubes a pale blue color, the seventh tube lilac or purple, and the remaining tubes no change, the reaction would be read as follows: 5555442000.

The Results of the Test in Various Syphilitic Conditions.—The change in color in the various tubes will differ with different syphilitic conditions of the central nervous system, the color reaction with paresis being the most characteristic and diagnostic of all, and the changes observed are called "curves." Thus, we have a "paretic curve" and a "luetic zone curve," as well as a "meningitic zone curve."

The "Paretic Curve."—The reaction observed in the cerebrospinal fluid in paresis is so constant and typical that it may be considered as the most valuable of all the confirmatory signs that we possess of this type of involvement of the central nervous system. It is to this type of the reaction that Miller and Levy[131] gave the name "paretic curve" and it is this type of reaction that is met with in practically 100 per cent of cases of paresis. In reporting the colloidal gold test it is usually customary to record the reaction upon a chart and when this is done, the paretic curve will appear substantially as in Table LIV.

Table LIV

Illustrating Type of Reaction Obtained with the Colloidal Gold Test in Paresis (Paretic Curve)

Color reactions	Color No.	Dilutions of cerebrospinal fluid with 0.4 per cent NaCl									
		1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120
Colorless	5	•	•	•	•	•	•				
Pale blue	4							•			
Blue	3								•		
Lilac or purple	2									•	
Red-blue	1										•
No change	0										

Recorded in numbers this reaction would be thus reported: 5555554210. Often the first three, four, or five tubes are decolorized, while the remaining ones show some changes, as would be found in the following combinations: 5554432200; 5555422100, 5555542210, or as many as eight of the tubes may be completely decolorized. Curves of this type are characteristic of paresis and are not found in other syphilitic diseases of the central nervous system, although a few reports are on record of cases of meningitis and multiple sclerosis giving similar curves with this test. A paretic curve is also observed in taboparesis.

The Luetic-Zone Curve.—The luetic-zone curve is obtained in cases of cerebrospinal syphilis and tabes, as well as in the fluid in the tertiary stage of syphilis. In tabes the colloidal gold test usually gives a luetic-zone curve of No. 4 intensity, the precipitation being only partial in the first two or three tubes, complete in the next two or more tubes, and gradually shading off into normal. However, it should be distinctly understood that there is nothing characteristic in the curves noted in tabetic patients and there is no constancy in the type of curve observed, except that the "paretic curve" is never noted. Table LV illustrates the "luetic-zone curve" obtained in a case of tabes.

Recorded by numbers the above reaction would be thus reported: 4445554200. In cerebrospinal syphilis the fluid usually shows weaker reactions than that for tabes but al-

TABLE LV

ILLUSTRATING TYPE OF REACTION OBTAINED WITH THE COLLOIDAL GOLD TEST IN TABES (LUETIC ZONE CURVE)

COLOR REACTIONS	COLOR NO.	DILUTIONS OF CEREBROSPINAL FLUID WITH 0.4 PER CENT NACL									
		1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120
Colorless	5										
Pale blue	4										
Blue	3										
Lilac or purple	2										
Red-blue	1										
No change	0										

ways in the luetic-zone, while the reactions obtained in the fluid during the tertiary stage of syphilis when symptoms of involvement of the central nervous system are absent are still weaker, only slight changes being observed, as a rule, in the luetic zone of the test.

In nonsyphilitic disease of the central nervous system, as in meningitis, acute poliomyelitis, multiple sclerosis, etc., the findings with the colloidal gold test are often confusing and of little practical value. In meningitis the so-called "meningitic zone" curve is encountered, in which the color changes occur in the highest dilutions, complete precipitation occurring at the opposite end of the chart from paresis. Table LVI illustrates the type of curve obtained in tuberculous meningitis or purulent meningitis.

TABLE LVI

ILLUSTRATING TYPE OF REACTION OBTAINED WITH THE COLLOIDAL GOLD TEST IN MENINGITIS (MENINGITIC CURVE)

COLOR REACTIONS	COLOR NO.	DILUTIONS OF CEREBROSPINAL FLUID WITH 0.4 PER CENT NACL									
		1-10	1-20	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120
Colorless	5										
Pale blue	4										
Blue	3										
Lilac or purple	2										
Red-blue	1										
No change	0										

Recorded by numbers the above reaction would be thus reported: 0001223550.

Interpretation of the Results of the Colloidal Gold Test.—There is only one condition in which the colloidal gold test may be practically relied upon for diagnosis and that is paresis. In the overwhelming number of instances, if a typical paretic curve is obtained with this test it is safe to make a diagnosis of this condition and the Wassermann test upon the blood and cerebrospinal fluid will generally confirm the diagnosis. However, the writer is still doubtful if we should rely upon the colloidal gold reaction alone in making a diagnosis of paresis, especially if the other diagnostic aids that we possess do not confirm the reaction. It must be admitted, however, that the first evidence we have in many cases of incipient paresis is a positive result with this test and whenever such a result is obtained it should be given great weight and the most intensive specific treatment at once instituted.

In cerebrospinal syphilis and tabes the colloidal gold reaction is of no diagnostic importance and the results obtained should never be considered as more than confirmatory of the diagnosis in cases which are very suspicious in other respects. With the exception of paresis and taboparesis the colloidal gold test is of value only as an aid in the diagnosis of syphilis of the central nervous system. In congenital syphilis, cerebrospinal syphilis, tabes, and secondary and tertiary syphilis the results are inconstant and only of confirmatory value, but its appearance in paresis, in many cases, before symptoms of the condition develop, renders the test of great value in early diagnosis and as this complication may occur in any case of syphilitic infection the routine testing of the cerebrospinal fluid with the colloidal gold test is to be encouraged.

The Cell Count

It is well known that in most inflammatory conditions of the central nervous system there is an increase in the number of cells found in the cerebrospinal fluid and this

increase occurs to such an extent in some conditions as to be of considerable service in their differential diagnosis. The cell count, however, so far as syphilitic disease of the central nervous system is concerned, is seldom of more than confirmatory value of results obtained by other methods of laboratory diagnosis. In no syphilitic condition of the central nervous system is the cell count specific nor can one differentiate syphilitic disease from other diseases of this system by its means.

An increase in the number of cells in the cerebrospinal fluid, called pleocytosis, occurs in practically 95 per cent of syphilitic affections of the central nervous system but also occurs in other diseases of this system, and in acute infections, as cerebrospinal meningitis, although in non-syphilitic conditions the cell count is seldom as high as in conditions like tabes, cerebrospinal syphilis, paresis, and syphilitic meningitis. According to Nonne[132] the cell count in the cerebrospinal fluid may be appreciably increased in from 30 to 40 per cent of cases of syphilis even in the absence of symptoms of organic nervous disease. In old cases of tabes, or cases in which the symptoms are not progressive, there may be an absence of pleocytosis and even in progressive cases this may sometimes occur.

Efforts have been made to differentiate the kinds of cells increased in the pleocytosis of syphilitic disease of the central nervous system, and Alzheimer has rendered it possible to recognize the different types of cells that occur. However, from a practical standpoint, the differentiation of the various cells found is of very little, if any, assistance in differentiating the various syphilitic diseases of the nervous system, a simple count of the cells present being as useful as any differential count. As Nonne well says:[133] "The attempt to recognize in the spinal fluid a characteristic cell-picture for the development of a paresis, or to foretell the time of progression of a syphilogenetic affection, has not as yet been crowned with success."

The cells which are increased in syphilitic disease of the central nervous system comprise both lymphocytes and leucocytes, but their origin is not absolutely determined. Whether they originate from the blood or from the connective tissue of the leptomeninges is unsettled but the pleocytosis is the direct result, undoubtedly, of an inflammatory process in the leptomeninges. This is the conclusion reached by Nonne and it is believed that it is the one generally held by the best students of the subject.

Technic of Counting the Cells.—In counting the cells in the cerebrospinal fluid the most generally used method is with the Fuchs-Rosenthal counting chamber. The method of making the dilution of spinal fluid and counting is as follows: The staining fluid consists of 0.1 gram gentian violet; 2.0 grams glacial acetic acid; and 50 c.c. distilled water. This fluid is drawn up into the ordinary pipette used for counting the white cells of the blood, to the point marked I and then the cerebrospinal fluid drawn up to the point marked II. The pipette is now shaken very thoroughly for at least two minutes, when a drop of the mixture is placed on the counting chamber, a few drops being expelled from the pipette before doing so. All of the lymphocytes and leucocytes are counted in all the squares and the total number divided by three. Nonne regards from 0 to 5 cells as normal; 6 to 10 as borderland cases; and more than 10 cells as pathological.

The Fuchs-Rosenthal counting chamber is not necessary in making a count of the cells in the spinal fluid, as the Zappert, Turk, or Levy counting chamber may be used just as well, the spinal fluid being undiluted or, if the cells are very numerous, diluted as for making a white blood cell count.

The staining of the cells is not necessary if the fluid be diluted with a 0.5 per cent solution in distilled water of glacial acetic acid, when the Fuchs-Rosenthal chamber for counting is used, while if either the Turk or Zappert count-

ing chambers are used the fluid should be used undiluted, unless the fluid is purulent or contains an excessive number of cells.

The number of cells found in the cerebrospinal fluid in the various syphilitic diseases of the nervous system varies very greatly and no diagnostic data can be based upon the number of the cells alone. The *increase* in the number of cells is the important diagnostic feature, but this increase is not of such a nature that one can say that paresis exists in this case, and tabes in that. In illustration of this, Table LVII shows the counts observed in paresis, cerebrospinal syphilis, and tabes.

TABLE LVII

CELLS COUNTS IN THE CEREBROSPINAL FLUID OBSERVED IN PARESIS, CEREBROSPINAL SYPHILIS, AND TABES

PARESIS		CEREBROSPINAL SYPHILIS		TABES	
CASE NO.	COUNT	CASE NO.	COUNT	CASE NO.	COUNT
1	18	1	13	1	9
2	45	2	64	2	8
3	67	3	243	3	43
4	16	4	11	4	44
5	29	5	86	5	55
6	88	6	65	6	69
7	12	7	50	7	186
8	36	8	590	8	237
9	168	9	344	9	36
10	243	10	156	10	110
11	507	11	6	11	124
12	323	12	402	12	116

It will be seen from the above table that there is nothing characteristic in the number of cells found in the cerebrospinal fluid in the three conditions, the characteristic feature being that there is an increase above normal in all the cases cited.

Interpretation of Results of Count.—The increase in the number of cells in the cerebrospinal fluid, as has been stated, is due to inflammatory conditions of the leptomeninges, but an increase does not always indicate that the condition causing it is syphilitic. Of the syphilitic con-

ditions of the central nervous system the highest counts are generally observed in syphilitic meningitis, the count frequently exceeding one thousand cells; in tabes the counts are apt to be high, running into the hundreds; in paresis, the average count is not above 100, generally running between 20 and 60, but counts above 100 are often obtained, and the same statement is true of cases of cerebrospinal syphilis. However, the mere number of cells found is no criterion of the condition present, and the cell count has to be interpreted in conjunction with the clinical symptoms present and the results of the other methods of laboratory diagnosis in syphilis; i. e., the Wassermann test, the colloidal gold test, and the test for increase in proteins in the cerebrospinal fluid.

Tests for Increase in Globulins in the Cerebrospinal Fluid.—In syphilitic disease of the central nervous system, as well as in other organic diseases of this system, the protein content of the cerebrospinal fluid is generally increased, and this increase can be detected by tests which have been perfected for demonstrating an increased globulin content in this fluid. Three methods are in general use, known as the Nonne and Apelt method, the Noguchi method, and the Pandy method.

The Nonne-Apelt Globulin Test.—This test is thus described by Nonne:[134] "The simplest method of testing the cerebrospinal fluid for albuminous bodies is to search for an increase of globulin—the so-called 'Phase I' reaction, introduced by Apelt and myself. For this purpose there is added to a hot saturated solution of sulphate of ammonium, which has been permitted to cool, an equal amount of cerebrospinal fluid; 1 c.c. of each is quite sufficient. It is advisable to pour one liquid gently on top of the other; if the globulins are increased, there occurs a more or less distinct gray ring at the plane of contact. After this preliminary observation the mixture is well shaken and the result may be read off within three minutes. If it is dis-

tinctly opalescent or cloudy, we call it a positive 'Phase I' reaction."

In making the above test it is best to add the cerebrospinal fluid from a 1 c.c. pipette very slowly to the ammonium sulphate solution, as in this way the gray ring indicating increased globulin is rendered much more distinct than when one fluid is poured into the other. This method is still in general use but has been very largely displaced by the Noguchi and Pandy methods.

The Noguchi Butyric Acid Test for Globulin.—This method was devised by Noguchi,[135] is simple, and accurate, and the one that the writer has generally used in his laboratories. The method of making the test is thus described by Noguchi:[136]

"Two parts of the cerebrospinal fluid to be examined are mixed with 5 parts of a 10 per cent butyric acid solution in physiological salt solution, and are heated over a flame and boiled for a brief period. One part of a normal solution of NaOH is then added quickly to the heated mixture, and the whole boiled once more for a few seconds. The actual quantities of these three reagents that I prefer are 0.1 or 0.2 c.c. of the spinal fluid, 0.5 c.c. of the butyric acid solution, and 0.1 c.c. of the normal sodium hydrate. It is necessary to take the precaution to employ for this test only cerebrospinal fluid entirely free from blood."

A positive reaction with this test is indicated by the appearance of a granular or flocculent white or gray precipitate which settles slowly to the bottom of the test tube. The amount of this precipitate and the rapidity with which it is formed will vary, as Noguchi observed, with the quantity of protein in the fluid examined, the greater the amount of protein the quicker the reaction will appear and the greater the amount of precipitate, in the one case the reaction appearing within a few minutes, while, if the amount of protein increase is small the reaction may take

an hour or more to appear. Two hours should be the time limit for the reaction.

The Pandy Method for Globulin Increase.—A method which is spoken very highly of by those who have used it is the Pandy method. It is the most simple of all methods that have so far been devised for the purpose and is undoubtedly reliable. Miller,[137] who has had a large experience with this method thus describes its use:

"Pandy's test has not received the attention it deserves. None of the other reactions used to reveal an excess of globulin is so simple in execution or so quickly decisive in its results. The reagent consists of a saturated aqueous solution of carbolic acid; ten parts of pure crystals are added to 100 parts of hot distilled water; the mixture is kept at room temperature for three or four days, during which time it should be frequently shaken. At the end of this time the clear supernatant fluid is drawn off into another bottle. To approximately 1 c.c. of the reagent is added one drop of the spinal fluid. Normally no change occurs or at most an extremely faint opalescence; with a fluid abnormal in its protein content there develops instantly at the point of contact a bluish white cloud, often resembling a ring of smoke, which gradually settles to the bottom of the tube."

The writer has had very little experience with this test but it has proved accurate in those cases in which it has been used.

Interpretation of the Results of the Globulin Tests.—In organic disease of the central nervous system an increase in the protein content of the cerebrospinal fluid commonly occurs and this increase is present in the vast majority of cases of syphilitic disease of this system. However, as this protein increase, indicated by the increase in globulins, is not confined to syphilitic disease, a positive reaction with any of the tests mentioned does not prove that the condition present is syphilitic nor is it possible to dif-

ferentiate the various syphilitic diseases of the central nervous system by their use. The tests are often positive in infections of the meninges due to the pneumococcus, tubercle bacillus, influenza bacillus, meningococcus, and in acute poliomyelitis, as well as in syphilitic disease, and are also present in chronic organic disease of the central nervous system, so that a positive reaction can only be regarded as confirmatory of the results of other laboratory methods of diagnosis of syphilitic involvement of this system and of the nature of the clinical symptoms which may be present.

Summary of the "Four Tests."—In diagnosing and in differentiating syphilitic disease of the central nervous system, it is necessary to carefully consider the results of the "Four tests" as they may be called, upon the cerebrospinal fluid. These are 1, the Wassermann test upon the fluid; 2, the colloidal gold test; 3, the cell count; 4, the test for increase in globulins. As an illustration of the results that are commonly obtained with these four tests, Table LVIII, adapted from Miller[138] is inserted:

TABLE LVIII

SHOWING AVERAGE FREQUENCY OF THE VARIOUS REACTIONS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

TEST	PARESIS %	TABES %	CEREBROSPINAL SYPHILIS %
Spinal fluid Wassermann	97	60-89	85-90
Globulin test	100	90-95	90-95
Cell count	98	85-90	85-90
Colloidal gold test	98-100	85-90	85-90
	Paretic curve	Luetic curve	Luetic curve

The blood Wassermann in these diseases gives approximately the following results: Paresis, 98-100 per cent; tabes, 70 per cent; and cerebrospinal syphilis, 70-80 per cent. A careful study of the cerebrospinal fluid by means of these four reactions will often result in enabling one to differentiate the various syphilitic diseases of the central

nervous system and, in the vast majority of our cases, if the tests are negative, to exclude syphilitic disease in a suspected individual. If the globulin test is negative in a patient suffering from disease of the central nervous system it is almost certain that the condition is not syphilitic, although, if positive, it can not be said that it is syphilitic. A fluid showing a positive Wassermann reaction in amounts as small as 0.2 c.c., together with a paretic curve with the colloidal gold test, is sufficient upon which to base a diagnosis of paresis, while a high cell count, a luetic curve with the colloidal gold test, and a negative Wassermann, or one only positive in large amounts of the spinal fluid, would point to tabes. By a careful comparison of the results of these various tests with the clinical symptoms present it should be possible, in the vast majority of instances, to make a diagnosis of the form of syphilitic disease of the central nervous system which may be present, and the value of these tests in diagnosing very early involvement of this system has already been emphasized.

In no case can it be stated that the syphilitic patient has been given the best that it is possible for medical science to give him, either in the way of diagnosis or treatment, if the thorough examination of the cerebrospinal fluid has been omitted and this examination should become as much of a routine in the diagnosis and treatment of syphilis as is the Wassermann test upon the blood serum.

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INDEX

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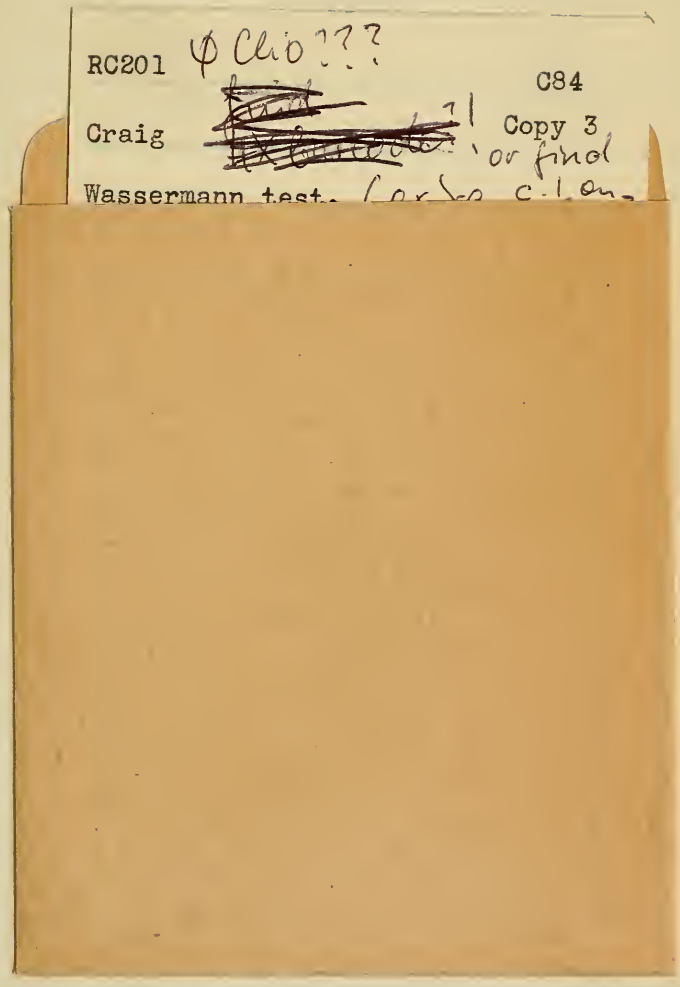
INDEX OF AUTHORS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